

FORM PTO-1300 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY DOCKET NUMBER UPN-3963
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLICATION NO. (if known see 37 C.F.R. 1.5) 09/720647
INTERNATIONAL APPLICATION NO. PCT/US99/15062	INTERNATIONAL FILING DATE 01 July 1999	PRIORITY DATE CLAIMED 01 July 1998 and 11 May 1999
TITLE OF INVENTION CAVITY INDUCED ALLOSTERIC MODIFICATION OF INTERMOLECULAR INTERACTIONS AND METHODS OF IDENTIFYING COMPOUNDS THAT EFFECT THE SAME		
APPLICANT(S) FOR DO/EO/US Ramachandran MURALI and Mark I. GREENE		
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:		
<ol style="list-style-type: none"> 1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. 2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. 3. <input checked="" type="checkbox"/> This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1). 4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date. 5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)). <ol style="list-style-type: none"> a. <input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau). b. <input type="checkbox"/> has been transmitted by the International Bureau. c. <input checked="" type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US) d. <input type="checkbox"/> is a translation of the International Application into English (35 U.S.C. 371(c)(2)). 6. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)) <ol style="list-style-type: none"> a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau). b. <input type="checkbox"/> have been transmitted by the International Bureau. c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. d. <input checked="" type="checkbox"/> have not been made and will not be made. 7. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)). 8. <input type="checkbox"/> An oath or declaration of the inventor(s) 35 U.S.C. 371(c)(4). 9. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)). 		
Items 11. to 16. below concern other document(s) or information included:		
<ol style="list-style-type: none"> 11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98. 12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. 13. <input type="checkbox"/> A FIRST preliminary amendment. 14. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment. 15. <input type="checkbox"/> A substitute specification. 16. <input type="checkbox"/> A change of power of attorney and/or address letter. 17. <input checked="" type="checkbox"/> Other items or information: <ul style="list-style-type: none"> - A copy of the application as published by WIPO under No. WO00/01349 including the Search Report - A copy of the International Preliminary Examination Report. 		
<p>EXPRESS MAIL Mailing Label No. EL659801351US Date of Deposit: December 28, 2000 I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231</p> <p>MAILER <u>Robert Galonsky</u> SIGNATURE <u>Robert Galonsky</u></p>		

U.S. APPLICATION NO. (37 CFR 1.53)

09/720647

INTERNATIONAL APPLICATION NO.
PCT/US99/15062ATTORNEY DOCKET NUMBER
UPN-3963

17. The following fees are submitted:

Basic National Fee (37 CFR 1.492(a)(1) - (5)):

Neither international preliminary examination fee (37 CFR 1.482)

nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO

and International Search Report not prepared by the EPO or JPO.....\$1,000.00

International preliminary examination fee (37 CFR 1.482 not paid to USPTO
but International Search Report has been prepared by the EPO or JPO.....\$860.00International preliminary examination fee (37 CFR 1.482) not paid to USPTO but
international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....\$710.00International preliminary examination fee paid to USPTO (37 CFR 1.482) but
all claims did not satisfy provisions of PCT Article 33(1)-(4).....\$690.00International preliminary examination fee paid to USPTO (37 CFR 1.482) and
all claims satisfied provisions of PCT Article 33(1)-(4).....\$100.00**ENTER APPROPRIATE BASIC FEE AMOUNT = \$690.00**Surcharge of \$130.00 for furnishing the oath or declaration later than _ 20 _ 30 months from
the earliest claimed priority date (37 CFR 1.492(e)).

Claims	Number Filed	Number Extra	Rate	
Total claims	5- 20 =	0	X \$18.00	\$
Independent Claims	5- 3 =	2	x \$80.00	\$160.00
Multiple dependent claims(s) (if applicable)			+ \$270.00	\$

TOTAL OF ABOVE CALCULATIONS = \$850.00Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are
reduced by 1/2.**SUBTOTAL = \$850.00**Processing fee of \$130.00 for furnishing the English translation later than _ 20 _ 30 months
from the earliest claimed priority date (37 CFR 1.492(f)).**TOTAL NATIONAL FEE = \$850.00**Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be
accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property**TOTAL FEES ENCLOSED = \$850.00**Amount to be:
refunded \$

charged \$

a. ☒ A check in the amount of \$ 850.00 to cover the above fee is enclosed.b. ☐ Please charge my Deposit Account No. 23-3050 in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.c. ☒ The Commissioner if hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account
No. 23-3050. A duplicate copy of this sheet is enclosed.**NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must
be filed and granted to restore the application to pending status.**

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33,229

REGISTRATION NUMBER

**CAVITY INDUCED ALLOSTERIC MODIFICATION OF
INTERMOLECULAR INTERACTIONS AND METHODS OF IDENTIFYING
COMPOUNDS THAT EFFECT THE SAME**

ACKNOWLEDGMENT OF GOVERNMENT RIGHTS

- 5 The present invention was made under Grant 1R21RR13360-01 from the National Institutes of Health. The Government may have certain rights to the invention.

FIELD OF THE INVENTION

- The present invention relates to the identification of compounds that modulate intermolecular interactions by allosterically modifying a functionally critical site
10 of a protein involved in such interactions and to methods of identifying the same.

BACKGROUND OF THE INVENTION

 This application claims priority to provisional application S.N. 60/091,431, filed July 1, 1998 and S.N. 60/133,435, filed May 11, 1999, which both have the same title as this application and which are both incorporated herein by reference.

- 15 One of the challenges in the development of therapeutic compounds is to find a small molecule that is able to mediate a desired biological effect. Traditionally, synthetic chemistry and natural product screening have been the principal means for the derivation of many drug products.

- High-throughput random screening is a standard procedure adopted by
20 pharmaceutical companies for the discovery of lead compounds. This method relies upon availability of a large chemical database of natural/medicinal products. This procedure does not require the knowledge of principle components of biomolecules that cause

disease. In short, it is a blind process to screen therapeutic lead compounds. The advantage of this approach is that it facilitates to build a large medicinal chemical database and can be repeatedly used to screen therapeutic compounds. Unfortunately, random screening is tedious and often requires isolation and characterization from natural extracts.

- 5 Natural products are complex and include the stereochemical complexities inherent in their natural origin.

While high-throughput random screening procedures have been used to identify some novel therapeutic molecules, such procedures are often limited by the availability of large chemical databases. Advances in computer technology and the
10 understanding of protein-protein interactions has allowed for attempts to replace the high-throughput screening procedures with computer-aided analysis and design of novel molecules. Such structure based approaches have reduced the time and resources to discover novel compounds.

- Structure based approaches have been used to develop several inhibitors
15 that are either "substrate analogs" or "allosteric" inhibitors. Allosteric effectors, in some cases, are considered superior to conventional substrate analog for reasons: (1) it is non-competitive with natural ligand, (2) it can be effective at a lower concentration, (3) allosteric binding sites are less conserved and thereby specificity and selectivity can be enhanced, and (4) in some cases allosteric effectors can inhibit the target molecules'
20 function by trapping it in an intermediate non-native or molten globular state.

Structure-based approaches represent a targeted pathway where therapeutic agents are designed towards the biomolecule responsible for disease. There are two major approaches in the design of lead therapeutic compounds based on the nature of the molecule. For enzymes, design of substrate analogs (from the knowledge of active site)
25 and peptidomimetics that has shown promise in some cases.

- Substrate analogs are developed to compete with the natural substrate and occupy the active site. Thus, a potent therapeutic compound must have high affinity, exhibit selectivity and have longer retention time. Substrate analogs are better suited for enzymes, because many receptors and other non-enzyme molecules, such as receptors and
30 their ligands have no defined active site but alter biological function. In such cases, a

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peptide's ability to mimic a protein's local structural features is one of the ways used to design therapeutic compounds. Substrate analog interactions are often not reversible.

- Peptidomimetics are developed both as therapeutic agents and as a probe to understand biological functions. Natural products targeting opioid and hormone receptors are historical examples of peptidomimetics because they validate many of the concepts invoked in rational design. These compounds provide a classic example of how structurally different non-peptides may be from their peptide parents (lacking flexibility, amide bonds and obvious pharmacophore similarity) and how their modification can lead to highly selective ligands for subtypes of receptors for both peptide and non-peptide compounds.

- Elucidation of the conformation of a peptide can provide insights about the structural requirements of its binding to a receptor (Boteju, L.W. et al., 1996, J. Med. Chem., 39:4120-4; and Cho, M.J. et al., 1996, Trends in Biotechnology, 14:153-8; which are both incorporated herein by reference). A major problem, however, in structure-activity studies of linear peptides is the large degree of flexibility, not only of the side-chain residues, but also of the peptide backbone. Substitution of individual amino acids followed by biological screening might reflect affective differences on structure rather than on residues implicated in binding. Consequently, spectroscopic studies in solution, where a rapid equilibrium between numerous conformations is likely to occur, have had little impact on the design of linear peptide analogues. In contrast, constrained peptides delineate solution conformations for correlation with receptor bound conformations. Bioactive compound design based upon conformational constrained peptide analogs representative of the recognition elements of the protein constitutes an effective approach to mimetic drug design. Constraints imposed upon peptides to lock in a particular conformation often times emulate those imposed by the tertiary structure of protein ligands. Imposed constraints can reflect the use of amino acids that contribute to the propensity of a particular secondary structure such as amphipathic helical repeats.

- Despite the diverse usefulness of peptidomimetics, they remain less viable drugs due to their poor bioviability. Nevertheless, active peptide analogues with modified bonds or side chains, provide another approach in defining bioactive conformations and

are valuable pharmacological probes, because generally they are more resistant to proteolytic degradation.

Protein structures have been elucidated using crystallography, NMR and molecular modeling. The three dimensional structures of proteins reveal (1) overall

- 5 folding of the molecule, (2) scaffolds: secondary structural features such as α -helix, β -sheet, (3) functional units; b-turns and loops, and (4) surfaces that include cavities, clefts, pockets and crevices formed by the folding of amino acid chains on itself and, in the case of multimeric protein complexes, on itself and the amino acid chains of other subunits. Cavities, clefts, pockets and crevices can accommodate water molecules within an interior.
- 10 Depending upon the nature of the amino acids which form the cavities, clefts, pockets and crevices molecules, the interior of these structural features have specific chemical and electrostatic properties as well as spatial dimensions.

Determination of crystal structures of proteins/receptors have provided a basic understanding of protein/receptors' function. Several receptors such as EGF

- 15 receptors are activated either by ligands or by association with other *erbB* family of receptors. One of the hypothesis is that conformational changes induced either by ligand or by co-receptors elicits signal transduction. Thus, it is presumed that through allosteric mechanisms receptors can modulate signal transduction. Allosterically driven biological functions are also known both in enzymes and receptors (Ellis, J., 1997, *Drug. Dev. Res.*, 40:193-204, and Kundrot, C.E. et al., 1991, *Biochem.*, 30:1478-1484, which are both incorporated herein by reference). Attempts to modulate the function of proteins/receptors have been made and often referred to as "allosteric modification or allosteric inhibitors".
- 20

Allosteric modification is a well known technique that has been studied in several enzymes (Iverson, L.F. et al., 1997, *Protein Science*, 6:971-982; Ladjimi, M.M. et

- 25 al., 1985, *J. Mol. Biol.*, 186:715-724; Ozaita, A. et al., 1997, *Brit. J. Pharm.*, 121:901-912; Tang, J. et al., 1997, *Chemistry & Biol.*, 4:453-459; and Tijane, M. et al., 1989, *FEBS Lett.*, 245:30-34; which are each incorporated herein by reference) and receptors (Berthold, M. et al., 1997, *Neurochem. Res.*, 22:1023-1031; Ellis, J., 1997, *Drug. Dev. Res.*, 40:193-204; Kolliasbaker, C.A. et al., 1997, *J. Pharmco. Exp. Therap.*, 281:761-768;
- 30 and Robichon, R. et al., 1997, *Eur. J. Pharmco.*, 328:255-263 which are each incorporated herein by reference). Hitherto techniques often used mutagenesis or small molecules

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identified from screening. Allosteric modifications have been used in enzymes to alter the enzymes' kinetics and in some cases used to develop inhibitors.

There is a need for modulators of intermolecular interactions and for methods of identifying such modulators. There is a need for inhibitors of intermolecular
5 interactions and for methods of identifying such inhibitors. There is a need for enhancers of intermolecular interactions and for methods of identifying such enhancers. Structure based ligand design, as practiced today, requires the knowledge of cavity of known functions such as active sites, or cavities identified by high throughput (ligand binding). There is a need for a generalized approach to identify functional cavities for novel ligand
10 design.

SUMMARY OF THE INVENTION

The present invention relates to methods of identifying compounds that modulate intermolecular interactions between a protein target and a modifier. Modulators may be inhibitors, i.e. compounds that inhibit intermolecular interactions, or enhancers,
15 i.e. compounds that enhances intermolecular interactions. According to the methods of the present invention, a cavity, cleft, pocket or crevice in the protein target which is proximal to a functionally critical site of the target protein involved in intermolecular interactions with the modifier is identified that may be distinct and proximal from the catalytic site. The volume of the cavity, cleft, pocket or crevice is calculated and its
20 chemical and electrostatic properties are mapped. Functional groups and compounds are identified which can be accommodated by the cavity, cleft, pocket or crevice. The parameters for identifying such functional groups and compounds include size, charge and hydrophobicity/hydrophilicity characteristics. Compounds which contain functional groups that can be accommodated by the cavity, cleft, pocket or crevice, including
25 compounds which can be completely accommodated by the cavity, cleft, pocket or crevice, are then tested in an *in vitro* assay to determine whether they modulate target-modifier interactions.

The present invention relates to pharmaceutical compositions and methods of treating an individual suffering from an inflammatory condition.

The present invention relates to pharmaceutical compositions and methods of treating an individual suffering from an undesirable immune response or immunological condition are disclosed.

- The present invention relates to pharmaceutical compositions and methods of treating an individual suffering from a bacterial infection are disclosed.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A and 1B depict the structure of third domain of TNF receptor.

- Figure 1A shows the disposition of cystine-knot loops (WP9) and a cavity near the binding site. The portion of the molecule denoted with an arrow shows the loop that was used as a template to design peptidomimetic. Figure 1B shows inhibition of TNF α -induced cytolysis of L929 cells by the antagonistic peptides. Absorbance obtained with 1 mg/ml of ACT-D alone and with ACT-D and 50 pg/ml of TNF α were considered as 100 % survival and 100% cytotoxicity, respectively. The results indicate the means and standard deviations derived from three independent experiments.

- Figures 2A, 2B and 2C show a preliminary result from a small database search in the third domain of TNF receptor. For clarity, only the domain of the receptor is shown. Figure 2A depicts the WP9 cavity of TNF receptor. Figure 2B shows the molecule s7 forms a complex with a binding energy of -40Kcal/mol without any chemical optimization. Since this compound is not chemically altered for maximal binding, kinetics of ligands have not been performed. Figure 2C shows results when tested in an apoptosis assay similar to the peptidomimetics, i.e. about 20% protection at 300 μ M concentration.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

- As used herein, the term "target protein" is meant to refer to a protein that is involved in intermolecular interactions with a modifier. The target protein may be a cellular protein or a protein that exists outside of a cell. The target protein may be, for example but without limitation to, a membrane bound protein, a cytosolic protein, a nuclear protein, an enzyme, a cytokine, a lymphokine, a chemokine, an adhesion molecule, a growth factor, or a receptor for such proteins. In some embodiments, the target protein is tumor necrosis factor (TNF) receptor family including TNF receptors, fas,

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CD40, gp30, and fas ligand, TNF α , CD4, β -lactamase, c-erbB2 p185 translation product, growth hormone receptor, growth hormone, insulin receptor, insulin, IL-1 receptor, IL-1, IL-2 receptor, IL-2, epidermal growth factor receptor (EGFR), and epidermal growth factor (EGF). A target protein must have a cavity, cleft groove pocket or crevice as part
5 of its three dimensional structure.

As used herein, the term "modifier" is meant to refer to a compound which is involved in intermolecular interactions with a target protein. The modifier may a proteinaceous molecule such as a protein, polypeptide or peptide, or a non-proteinaceous molecule such as a sugar, polysaccharide, nucleic acid molecule, or other non-
10 proteinaceous organic or non-organic molecule. The term modifier may be used interchangeably herein with the term "ligand". Examples of proteinaceous modifiers include: proteins such as membrane bound proteins, cytosolic proteins, and nuclear proteins; and proteins, polypeptides and peptides such as proteinaceous enzyme substrates, cytokines, lymphokines, chemokines, adhesion molecules, growth factors, or receptors for
15 such molecules. In some embodiments, the modifier is (TNF) receptor family including TNF receptors, fas, CD40, gp30, and fas ligand, TNF α , CD4, β -lactamase, c-erbB2 p185 translation product, growth hormone receptor, growth hormone, insulin receptor, insulin, IL-1 receptor, IL-1, IL-2 receptor, IL-2, epidermal growth factor receptor (EGFR), and epidermal growth factor (EGF).

As used herein, the term "intermolecular interactions" is meant to refer to interactions that occur between and protein, which is referred to as the target protein, and a second molecule, which is referred to as a modifier. The interactions occur at a site on the target protein referred to as the target protein:modifier interaction site. Intermolecular interactions include for example: association, oligomerization, binding, and
25 conformational/structural perturbances. The intermolecular interaction between the target protein and the modifier results in some biological activity, the enhancement or inhibition of which is desirable in some circumstances. Examples of intermolecular interactions which result in a biological activity include processing of substrates by enzymes, ligand induced signal transduction, allosteric modulators, signal transduction due
30 oligomerization, and protein small molecule binding (antagonists/agonists).

As used herein, the term "target protein:modifier interaction site" is meant to refer to the location on the target protein in which interaction between the target protein and the modifier occurs. In examples where the target protein is an enzyme and the modifier is an enzyme substrate, for example, the target protein:modifier interaction site is also referred to as the catalytic site. In examples where the target protein is a receptor, for example, the target protein:modifier interaction site is also referred to as the binding site. In some cases, such as when the target protein is a member of the immunoglobulin superfamily, the target protein:modifier interaction site may include complementarity determining regions (CDRs) or loops, which define the portions of the target protein which interact directly with modifier.

As used herein, the terms "cavity", "cleft", "pocket", "groove" and "crevice" are used interchangeable and are meant to refer to a molecular surface or location on a target protein that can accommodate at least one solvent such as for example water molecules, although some cavities may not be solvated. The identification process involves using molecular models in which a spherical probe of radius 1.4 Å, which is approximate to a water molecule, is used to track the surface of the molecule. A cavity, can accommodate a water molecule, i.e. the probe that is the equivalent size of a water molecule can fit within the cavity. Accordingly, a cavity has dimensions and a volume which can be measured.

As used herein, the term "functionally critical site" is meant to refer to a site or region or location or secondary structural element on a target protein that is involved in either altering or mediating a function, the modulation of which is desirable. According to some embodiments of the invention, the function can be processing of a modifier that is an enzyme substrate by a target protein that is an enzyme, the functionally critical site is a target protein:modifier interaction site that is a catalytic site, and the desirable modulation is the inhibition of substrate processing by the enzyme. According to some embodiments of the invention, the function can be binding of the modifier to the target protein, the functionally critical site is a target protein:modifier interaction site that is a binding site, and the desirable modulation is the inhibition of target protein-modifier binding. Other examples of functionally critical sites include surfaces of a target protein which interface with an oligomer and loops that stabilize oligomers.

As used herein, the term "proximal" is used interchangeably with the term "adjacent to" and is meant to refer to the distinct locations of the cavity and a functionally critical site which is at a measurable distance. According to the invention, the cavity is at a distinct location from the functionally critical site. The two locations are distinct from each other so that the modification of the functionally critical site that occurs when a functional group of a compound occupies the cavity is allosteric modification. A cavity is proximal to a functionally critical site if the functionally critical site can be altered by molecular interactions between the target protein and at least a functional group of a compound which can be accommodated by the cavity. In preferred embodiments, a cavity that is proximal to a functionally critical site is generally within about 15-20 Angstroms to the functionally critical site.

As used herein, the term "modulate" is meant to refer to an effect upon intermolecular interactions may be caused by compounds according to the invention which allosterically modify molecular surfaces involved in such intermolecular interactions. Such compounds are referred to herein as "modulators". In some embodiments, the effect caused by a modulator may be the inhibition of intermolecular interactions, in which case the modulator is an "inhibitor". In some embodiments, the effect caused by a modulator may be the enhancement of intermolecular interactions, in which case the modulator is an "enhancer".

According to the invention, modulators, such as inhibitors or enhancers, of intermolecular interactions may be identified or designed to allosterically modify molecular surfaces involved in such intermolecular interactions. Thus, any intermolecular interactions between a target protein that has a cavity proximal to a functionally critical site such as a binding site or catalytic site, and a second molecule, a modifier which may or may not be a protein can be affected using compounds identified according to the invention.

The invention comprises a series of steps including: 1) identifying a cavity proximal to a functional critical site; 2) determining physical parameters of the cavity, 3) identifying functional groups which can be accommodated by the cavity; and 4) testing compounds which comprise such functional groups in an *in vitro* assay to determine whether such compounds are active.

According to the invention, the target protein must interact with a modifier and have a cavity proximal to a functional site. By identifying functionally critical sites and cavities of a target protein or modifier, which can be done routinely, it has been discovered that such cavities, if proximal to the functionally critical site, can be targets for

5 compounds that can modulate the activity of the target protein with respect to its interaction with modifiers. Since the interaction with modifiers is necessary for a specific biological function attributed to the target protein, inhibition of target protein:modifier interaction inhibits the biological function associated with such interaction. Likewise, the enhancement of target protein:modifier interaction may enhance the biological function

10 associated with such interaction.

The means to identify functionally critical sites on a target protein are numerous, varied and well known. For example, the identification of active or catalytic sites of enzymes, and the binding sites of receptors or ligands are well known. The functionally critical site of a target protein may be identified several different ways

15 including, but not limited to: by identification of β -factors on the target protein structure as imaged using crystal or nuclear magnetic resonance (NMR) images either by thermal β -factors on the atoms of target protein from crystal structure or flexible loops inferred from NMR signals, or microcalorimetric analysis of complex or mutation analysis of molecule, by protein, peptide or peptidomimetic mapping of the target protein including

20 immunomapping; and by identifying CDRs on the target protein structure. β -factors are parameters that define flexibility such as thermal parameters from crystallographic studies. Thermal β -factors are parameters that reflect the disordered (flexible) nature of atoms in the 3D structure determined by X-ray diffraction. When the structures are determined by X-ray diffraction, the data needed to determine β -factors are measured as diffracted

25 intensities. Fourier transform analysis of these data reveal the β -factors associated with the atoms in the molecule and β -factors always determined as a part of the crystal structure analysis. These β -factors reflect the disorder or flexibility of the atoms in the molecule. Calorimetric values from thermodynamic studies can also be used to identify functionally critical site of a target protein. An algorithm has been described which is also useful to

30 identify mobile regions. This algorithm and its use are described in Daquino, J.A. et al., 1996, Proteins, 25:143-156; Gomez, J. et al., 1995, Journal of Molecular Biology,

252:337-350; Hilser, V.J. et al., 1996, J. Mol. Biol., 262:756-772; and Xie, D. et al., Protein Science, 3:2175-2184; which are each incorporated herein by reference.

- The cavity of the target protein may be identified by any of several well known techniques including, but not limited to, crystal structure analysis, NMR and
- 5 computer models. The cavity size must be able to accommodate at least one water molecule. The techniques for identifying cavities on the surface of proteins are well known and described for example in "*Protein Engineering*", Edited by Dale L. Oxender, C. Fred Fox, Liss Co., New York (1987) (for crystallography & NMR) and "*Guidebook on Molecular Modeling in Drug Design*", Edited by N. Claude Cohen, Academic Press,
 - 10 1996San Diego, Calif. (1996) (for computer modeling), which are each incorporated herein by reference. To determine whether a surface can accommodate water, using the computer model of the protein, the surface is probed with small sphere of radius 1.4Å, a size similar to that of a water molecule. The atoms touched by the probe sphere are marked as surface atoms. Mapping the surface atoms as a continuous surface defines the
 - 15 geometry of the surface. The geometry then allows one to classify cavities. To be proximal the cavity must not be at the same location as the functionally critical site.

- Once a cavity that is proximal to the functionally critical site is identified, certain physical parameters are ascertained. Such parameters include at least one and preferably more than one of the following: the volume and dimensions of the cavity are
- 20 measured or otherwise calculated; the electrostatic properties of the cavity and/or the chemical properties, i.e. hydrophobicity/hydrophilicity, of the cavity may be mapped. The interior of the cavity is thus defined by the volume and dimensions of the interior of the cavity and/or the map of electrostatic properties within the interior of the cavity and/or the map of chemical properties within the interior of the cavity.

- 25 In some embodiments, the volume and dimensions of the interior of the cavity can be determined by rolling a probe radius of 1.4 Å (equivalent of one water molecule) to generate a surface. The accessible surface is then calculated using among many other programs freely available the program MS (Michael S. Connolly). MS is available from QCPE (QCPE, Creative Arts Bldg., 181, Indiana University, Bloomington,
- 30 IN 47405) for and also packaged in several graphic software such as INSIGHT and QUANTA (both available from Molecular Simulations, Inc. San Diego, CA). In addition,

the program described in Kleywegt, G.J. et al., 1994, Acta, D50:178-185, which is incorporated herein by reference, can also be used to detect, measure and characterize cavities.

The electrostatic properties and chemical properties, i.e.

- 5 hydrophobicity/hydrophilicity, of the cavity can be mapped. The residues in the binding region are analyzed for site-points (atoms that are capable of forming hydrogen bonds, hydrophobic interactions) using the program GENSITES. Other equivalent programs such SPHGEN which is part of DOCK can also be used. The DOCK program is available from Prof. Kuntz laboratory, University of California at San Francisco, San Francisco CA.
- 10 The program identifies possible locations based upon differences in surface accessibility of different sized spheres rolling over the molecular surface of the target protein. A three dimensional map of the interior of the cavity is generated which corresponds to the dimensions, charge and chemical properties of the interior surfaces.

- Once physical parameters of the cavity are ascertained, functional groups
- 15 are identified which can be accommodated by the cavity. Such functional groups must be of an appropriate size such that they can fit within the interior of the cavity. Additionally, functional groups must be electrostatically and chemically compatible with interior of the cavity. That is, the functional group must have electrostatic properties and chemical properties which would result in forces that attract the functional group to the interior of
 - 20 the cavity rather than repelling forces which would inhibit or prevent the functional group from occupying the interior of the cavity. Using the site points developed in the cavity, possible molecular fragments are identified using the program LUDI. LUDI is part of INSIGHT which is available from Molecular Simulations, Inc. Another program from QUANTA called CAVEAT, also available from Molecular Simulations, Inc., can be used
 - 25 to identify functional groups which can be accommodated by the cavity.

- In some preferred embodiments, shape complementarity is used as initial
- screen for detecting fragments with different moieties. The molecular modeling approach assumes that the site is relatively rigid and that the intramolecular energy change upon target protein/modulator binding is small compared to the interaction energy between
 - 30 target protein/modifier conformations. Therefore, the binding mode specifies which molecular point (expressible as Cartesian coordinates) on the modulator should be bound

to which site point (also Cartesian coordinates) at the binding site. The fitting procedure is tantamount to identifying common surface features with subsequent docking of complementary surfaces.

- Docking between two complementary surfaces can be an exhaustive
- 5 procedure even with known topography. First, a probe sphere is rolled on the binding surface as the locus of the possible positions which can be occupied by the atoms of the binding molecule. This continuum of loci can be reduced to a set of discrete points localized at each residue and assigned a type. An additional type assignment for each site point is given depending on the relative geometric description of this residue with its three
- 10 closest neighbors. These points define regions for fitting fragments identified in the LUDI data base. Complexes are subjected to energy minimization and molecular dynamics calculations to optimize the relative orientations and to monitor conformational changes in the target protein that are induced upon complex formation. This procedure is done using AUTODOCK (Goodsell, et al. 1996 which is incorporated herein by reference) and
- 15 LIGIN (Sobolev, et al. 1996 which is incorporated herein by reference) or any other equivalent programs that use docking algorithms. These two methods allow exploration of both conformational flexibility and possible chemical modification for enhanced binding properties. This approach provides an estimate of the size of the molecule that can bind and identify possible functional groups that can interact with neighboring
- 20 residues and provides a way to develop novel molecular structures based on the distribution of site points.

- Novel molecular compounds based on site points may encounter difficulty in synthesis and suitability for biological assays. To overcome this obstacle, large three-dimensional chemical structure databases (MDL Corp., San Leandro, CA) are
- 25 searched to identify compounds (Good, A.C. et al., 1995, J. Comput. Aided Mol. Des., 9:1-12; Kuntz, I.D., 1992, *Science*, 257:1078-1082; and Li, S. et al., 1997, *Proc. Natl. Acad. Sci. U.S.A.*, 94:73-78; which are each incorporated herein by reference). The advantage of using the chemical database is two fold: (1) it offers a unique opportunity to search for novel molecules to small fragments that can be easily incorporated in a larger
- 30 compound and (2) selection of chemical compounds is facilitated from the knowledge of their availability, synthetic pathway, toxicity, and solubility etc. Currently, the

- three-dimensional structure chemical database contains about 250,000 small molecules. Therapeutically useful compounds can be identified in the chemical databases using the DOCK (Good, A.C. et al., 1995, J. Comput. Aided Mol. Des., 9:1-12 and Goodsell, D.S. et al., 1996, J. Mol. Recogn., 9:1-5, which are both incorporated herein by reference)
- 5 algorithm. The cavity is explored with each small molecule from the database for maximal interaction such as hydrophobic, hydrogen bonds and complement electrostatic properties by conformational search. Based on the binding energy, the molecules are ranked and, for example, the top 200 compounds are selected. In addition, molecules similar to the one constructed from *de novo* ligand design can be identified in the
- 10 databases using a three-dimensionally constrained fragment search. The short listed molecules obtained both by database search (DOCK) and fragment search are used to create a small chemical database library using MDL's project library software. Quantitative Structure Activity Relation (QSAR) analysis in medicinal chemistry and pharmacology has proven useful in making predictions for molecules that are chemically
- 15 similar to those of the original data set. Distance geometry directed QSAR allows for testing of a much wider class of compounds due to its independence from physico/chemical parameters. The molecules in the library are compared for a common motif and analysis similar to 3D-QSAR are carried out using ASP and TSAR (Oxford Molecular, Oxford, England) sequentially to find the suitable functional groups for
- 20 maximal binding energy.

- Following identification, compounds selected by one of the various approaches or combinations thereof are evaluated for biological activity in an *in vitro* assay to determine whether they modulate target-modifier interactions. Biological assays are utilized for which intermolecular interactions are known to result in a detectable signal
- 25 or phenotype or for which it is known that inhibition of intermolecular interactions result in a detectable signal or phenotype. Using such assays, comparative assays are performed in the presence or absence of the identified compounds to confirm biological activity of the compound.

- Pharmaceutical compositions according to the invention include
- 30 components identified by the methods of the invention which further comprise a pharmaceutically acceptable carriers or vehicles, such as, for example, saline. Any

medium may be used which allows for successful delivery of the compound. One skilled in the art would readily comprehend the multitude of pharmaceutically acceptable media that may be used in the present invention. The term "pharmaceutical" is well known and widely understood by those skilled in the art. As used herein, the terms "pharmaceutical compositions" and "injectable pharmaceutical compositions" are meant to have their ordinary meaning as understood by those skilled in the art. Pharmaceutical compositions, such as injectable pharmaceutical compositions, are required to meet specific standards regarding sterility, pyrogens, particulate matter as well as isotonicity and pH, i.e. *inter alia* sterile, pyrogen-free and free of particulate matter.

- 10 Pharmaceutical compositions may be formulated by one having ordinary skill in the art with compositions selected depending upon the chosen mode of administration. Suitable pharmaceutical carriers are described in *Remington's Pharmaceutical Sciences*, 18th Edition, A.R. Gennaro, Ed., Mack Publishing Company, Easton, PA, a standard reference text in this field, which is incorporated herein by
- 15 reference.

 The pharmaceutical compositions of the present invention may be administered by any means that enables the active agent to reach the agent's site of action in the body of a mammal. Pharmaceutical compositions may be administered parenterally, i.e., intratumor, intravenous, subcutaneous, intramuscular. Intravenous and

20 intratumor administration are preferred routes. For example, in cases where intramuscular injection is the chosen mode of administration, an isotonic formulation is preferably used. Generally, additives for isotonicity can include sodium chloride, dextrose, mannitol, sorbitol and lactose. In some cases, isotonic solutions such as phosphate buffered saline are preferred. Stabilizers include gelatin and albumin.

- 25 Dosage varies depending upon known factors such as the pharmacodynamic characteristics of the particular agent, and its mode and route of administration; age, health, and weight of the recipient; nature and extent of symptoms, kind of concurrent treatment, frequency of treatment, and the effect desired.

EXAMPLES

30 Example 1

In one embodiment of the invention, CIAM technology is used to identify compounds that inhibit interactions between tumor necrosis factor (TNF) receptor and TNF α .

- Tumor necrosis factor receptor is one of the first receptors to be studied at the atomic detail both as a complex and uncomplexed polypeptide. The crystal structure of the TNF receptor both in complexed and uncomplexed forms provides a general understanding by which these receptors bind to their ligands (Banner, D. et al., 1993, Cell, 73:431-45; Eck, M.J., et al. 1989, J. Biol. Chem., 264:17595-605; and Eck, M.J., et al. 1992, J. Biol. Chem., 267:2119-22; which are each incorporated herein by reference) and associated ligand induced conformational changes. The cystine knot in the TNF receptor family consists of 42 amino acid residues with 6 cystine residues forming three inter chain disulfide bond to create the structural motif. The three dimensional structure reveals the cystine-knots repeats about 30 Å in length are arranged in a head-to-tail fashion exposing the loops on one side of the receptor. These loops are either involved in oligomerization or ligand binding. Uncomplexed TNF receptors are observed as dimers. In the dimeric form, the first and last cystine domains involved dimeric contacts. The membrane proximal domain is disordered perhaps due to the lack of the transmembrane that normally holds this domain in a stable state. Crystal structure analysis of TNF receptor and TNF β complex shows that there are three distinct binding sites, referred to as "WP5", "WP8" and "WP9".

To understand, the most energetically relevant binding sites, peptides were used as probes and several cyclic peptides were developed for species.

- Peptidomimetics were developed and tested from all three surface loops of the TNF receptor: loop (56-73) of domain 1; loop (76-83) of domain 2 and loop (107-114) of domain 3 (Figure 1A). The peptidomimetics are described in detail in Takasai, et al. 1997 Nature Biotechnology 15:1266-1270, which is incorporated herein by reference. The peptidomimetic engineered from the third domain (WP9QY) inhibited TNF α binding (IC₅₀=75 mM) to its receptor. Also, the peptidomimetic protected cells against TNF α induced cell death when apoptosis was induced with 7 pg of TNF α suggesting that the peptide specifically bind to TNF α . The peptidomimetic (WP9QY) is one of the first peptides to show anti-TNF α activity (Figure 1B).

- Based on the effect of the small loop in the third domain of TNF receptor identified by peptidomimetic analysis, a large cleft was identified that could be utilized for docking an allosteric inhibitor. The cavity is shallow: 8 Å deep, 17.6 Å long and 12.4 Å wide (Figure 2A). The walls of the cavity are formed by residues involved in binding
- 5 TNFα. The large cleft close to one of the binding sites (WP9) was used to perturb these loops by an allosteric effect, using a small molecule designed from the above procedures (DesJarlais, R.L. et al., 1986, *J. Med. Chem.*, 29:2149-2153; DesJarlais, R.L. et al., 1988, *J. Med. Chem.*, 3:722-729; Good, A.C. et al., 1995, *J. Comput. Aided Mol. Des.*, 9:1-12; Gschwend, D.A. et al., 1996, *J. Mol. Recogn.*, 9:175-186; Shoicet, B.K. et al., 1991, *J.*
- 10 *Mol. Biol.*, 221:327-346; Strynadka, N.C. et al., 1996, *Nat. Struct. Biol.*, 3:233-239; and Strynadka, N.C. et al., 1996, *Nat. Struct. Biol.*, 3:290-297; which are each incorporated herein by reference). About 232 structurally suitable molecules were selected from an initial screening of small chemical database built using MDL (MDL corporation, San Leandro, CA) structural chemical database containing about 10000 structures. Further
- 15 analysis revealed that not all of them are conducive for biological experiments based on solubility and toxicity. For the purpose of testing, four compounds were tested for biological activity, but not for specificity and kinetics. The compounds were tested for apoptosis using a standard MTT assay (Hansen, M.B. et al., 1989, *J. Immunol. Meth.*, 119:203-210, which is incorporated herein by reference). One compound, S7 with
- 20 binding energy of -40Kcal/mol (Figure 2B) showed activity (Figure 2C) in the MTT assay. These results indicate that small molecules can be developed as pseudo-allosteric inhibitors.

- The present invention provides a novel strategy to modify the conformation of specific loops in an approach referred to as cavity induced allosteric modification
- 25 (CIAM) between the target protein, TNF receptor, and the modifier, TNF. This strategy uses the known crystal structure of the TNF receptor. The surface of the target protein... was generated by rolling a probe radius of 1.4 Å (equivalent of water molecule). The ... accessible surface was calculated using program MS (Connolly, M.L. et al., 1993, *J. Mol. Graph.*, 11:139-141 and Langridge, R. et al., 1981, *Science*, 211:661-666, which are both
- 30 incorporated herein by reference). The residues in the binding region are analyzed for site-points (atoms that are capable of forming hydrogen bonds, hydrophobic interactions)

using the program GENSITES. which identifies possible locations based upon differences in surface accessibility of different sized spheres rolling over the molecular surface of the target protein. Using the site points developed in the cavity, possible molecular fragments were identified using the program LUDI (Bohm, L.W. et al., J. Mol. Recogn., 6:131-137
5 which is incorporated herein by reference). Shape complementarity was used as initial screen for detecting fragments with different moieties. The molecular modeling approach assumes that the site is relatively rigid and that the intramolecular energy change upon ligand/inhibitor binding is small compared to the interaction energy between receptor/protein conformations. Therefore, the binding mode specifies which molecular
10 point (expressible as Cartesian coordinates) on the inhibitor should be bound to which site point (also Cartesian coordinates) at the binding site. The fitting procedure is tantamount to identifying common surface features with subsequent docking of complementary surfaces.

Docking between two complementary surfaces can be an exhaustive
15 procedure even with known topography. However, one can reduce the dimensionality of the problem by computing site points on the binding surface. First, a probe sphere is rolled on the binding surface as the locus of the possible positions which can be occupied by the atoms of the binding molecule. This continuum of loci can be reduced to a set of discrete points localized at each residue and assigned a type. An additional type
20 assignment for each site point is given depending on the relative geometric description of this residue with its three closest neighbors. These points define regions for fitting fragments identified in the LUDI data base. Complexes are subjected to energy minimization and molecular dynamics calculations to optimize the relative orientations and to monitor conformational changes in the ligand that are induced upon complex
25 formation. This procedure is done using AUTODOCK (Goodsell, D.S. et al., 1996, J. Mol. Recogn., 9:1-5 which is incorporated herein by reference) and LIGIN (Sobolev, et al. 1996 Proteins, 25:120-129 which is incorporated herein by reference). These two methods allow exploration of both conformational flexibility and possible chemical modification for enhanced binding properties. This approach provides an estimate of the
30 size of the molecule that can bind, identifies possible functional groups that can interact with neighboring residues, and provides a way to develop novel molecular structures

based on the distribution of site points. Often, novel molecular compounds based on site points encounter difficulty in synthesis and suitability for biological assays.

- To overcome this obstacle, large three-dimensional chemical structure databases (MDL Corp., San Leandro, CA) are searched to identify compounds (Good, A.C. et al., 1995, J. Comput. Aided Mol. Des., 9:1-12; Kuntz, I.D., 1992, Science, 257:1078-1082; and Li, S. et al., 1997, Proc. Natl. Acad. Sci. U.S.A., 94:73-78; which are each incorporated herein by reference). The advantage of using the chemical database is two fold: (1) it offers a unique opportunity to search for novel molecules to small fragments that can be easily incorporated in a larger compound and (2) selection of chemical compounds is facilitated from the knowledge of their availability, synthetic pathway, toxicity, and solubility etc. Currently, the three-dimensional structure chemical database contains about 250,000 small molecules. Therapeutically useful compounds can be identified in the chemical databases using the DOCK (Good, A.C. et al., 1995, J. Comput. Aided Mol. Des., 9:1-12 and Goodsell, D.S. et al., 1996, J. Mol. Recogn., 9:1-5, which are both incorporated herein by reference) algorithm. The cavity is explored with each small molecule from the database for maximal interaction such as hydrophobic, hydrogen bonds and complement electrostatic properties by conformational search. Based on the binding energy, the molecules are ranked and the top 200 compounds are selected. In addition, molecules similar to the one constructed from *de novo* ligand design can be identified in the databases using three-dimensionally constrained fragment search. The short listed molecules obtained both by database search (DOCK) and fragment search are used to create a small chemical database library using MDL's project library software. Quantitative Structure Activity Relation (QSAR) analysis in medicinal chemistry and pharmacology has proven useful in making predictions for molecules that are chemically similar to those of the original data set. Distance geometry directed QSAR allows for testing of a much wider class of compounds due to its independence from physico/chemical parameters. The molecules in the library are compared for a common motif and analysis similar to 3D-QSAR are carried out using ASP and TSAR (Oxford Molecular, Oxford, England) sequentially to find the suitable functional groups for maximal binding energy. Finally, compounds selected from different approaches are evaluated using biological activities.

Cytotoxicity assay

- The murine fibroblast cell line, L929 is maintained in Dulbecco's modified Eagle's medium supplemented with 10% FCS, and the medium is replaced with serum free AIM-V medium (GIBCO BRL) right before seeding of the cells for an assay. L929 cells
- 5 are seeded onto 96-well microtiter plates (2×10^4 cells/well), and incubated for 20 hr at 37°C under 5% CO₂ in air. After preincubation with actinomycin D (ACT-D) for 2 hr at a final concentration of 1 mg/ml, TNF α (7 pg) / inhibitor solution (100-80 ml), preincubated in PBS for 1 hr at 37°C, is added to the wells. The cells are incubated with TNF α finally adjusted to 50 pg/ml for 7 hr at 37°C under 5% CO₂, and stained with MTT (Sigma).
 - 10 Briefly, 10 ml of the 10 mg/ml solution of MTT is added to each well, and after 2 hr incubation at 37°C, the formazan formed is colored by overnight incubation at 37°C with 100 ml of extraction buffer (20% SDS in 50% DMF, pH 4.7). Finally the optical density of colored formazan is measured at 600 nm.

Competitive radioreceptor assay

- 15 TNF-receptor chimeric protein (100 ng/ml) diluted in PBS (100 ml) is immobilized onto MicroTest III flexible assay plate (Becton Dickinson, San Jose, CA) by an incubation at 4°C overnight. After blocking with PBS containing 1% bovine serum albumin (BSA) for 2 hr at room temperature and subsequent washing with PBS containing 0.1% Tween 20 (PBS-Tw), ¹²⁵I-labeled -TNF α (1ng)/inhibitor solution (100 ml)
- 20 preincubated in PBS for 1 hr at 37°C are added onto the TNF-receptor coated wells. After 2 hr incubation at 37°C, the plate is washed with PBS-Tw, and bound radioactivity is measured in Cobra gamma counter (Packard Instruments, Meriden, CT).

- In some embodiments, the pharmaceutical compositions of the present invention comprise compounds having Formula I which is set forth in the section below
- 25 entitled Formulae. In compound of Formula I, R₁ and R₂ are, independently, selected from the group consisting of -H, -OCH₃, -CH₂CH₃, -*t*-butyl, 3-carboxy-4-chlorophenylamino, -N-(CH₂CH₂OH)₂, and -O(O)C-Ph. R₃ is selected from the group consisting of -H, ethyl, -OCH₃, -Cl, Br, F, 3-carboxy-4-chlorophenylamino, -N-(CH₂CH₂OH)₂, -*t*-butyl, and -OC(O)-Ph, and is not limited to attachment at any certain
 - 30 position on the phenyl ring to which it is attached. Preferably, R₃ is attached at either the

1 or 4 position of the phenyl ring. R_4 is selected from the group consisting of -Br, -Cl, and -F.

In some preferred compounds of Formula I

- 5 R_1 , R_2 , and R_3 are -OCH₃, R_3 is attached at the 4 position, R_4 is -Cl;
- R_1 and R_2 are methyl, R_3 is ethyl, attached at the 4 position, R_4 is -Cl
- R_1 and R_2 are -OCH₃, R_3 is -Cl, attached at the 2 position, R_4 is -Cl,
- 10 R_1 and R_2 are -OCH₃ and R_3 is H, R_4 is -Cl;
- R_1 is H, R_2 and R_3 are 3-carboxy-4-chlorophenylamino, and R_3 is attached at the 4 position, R_4 is -Cl;
- R_1 and R_2 are -N(CH₂CH₂OH)₂, R_3 is Cl, attached at the 4 position, R_4 is -Cl;
- 15 R_1 , R_2 , and R_3 are *t*-butyl, R_3 is attached at the 4 position, R_4 is -Cl,
- R_1 is -OCH₃, R_2 and R_3 are H, R_4 is Cl; or
- R_1 , R_2 , and R_3 are benzoate, R_3 is attached at the 4 position, R_4 is -Br.

Some preferred compounds of Formula I have the structures I-A, I-B, I-C, I-D, I-E, I-F, I-G, I-H or I-I which are set forth below in the section entitled Formulae:

20 These compounds are available from the following suppliers:

	Compound	Catalog Number	Supplier
	I-A	F36,700-1	Aldrich, Milwaukee, WI
	I-B	S11,245-3	Aldrich, Milwaukee, WI
	I-C	00569	Ryan Scientific, Isle of Palms, S.C.
25	I-D	F10,001-3	Aldrich, Milwaukee, WI
	I-E	00129	George UHE, Paramus, NJ
	I-F	F37,166-1	Aldrich, Milwaukee, WI
	I-G	S-11,239-9	Aldrich, Milwaukee, WI
	I-H	F-27,721-5	Aldrich, Milwaukee, WI
30	I-I	F12,920-8	Aldrich, Milwaukee, WI

- In some embodiments, the pharmaceutical compositions of the present invention comprise compounds having Formula II which is set forth below in the section entitled Formulae. In compounds having Formula II, R_1 is selected from the group consisting of -diphenylchloro methyl, -di(4-chlorophenyl)chloro methyl, and 4-(diphenylchloromethyl)phenyl; and R_2 , R_3 , R_4 are independently selected from the group consisting of -Br, -Cl, and -F, and are preferably -Cl.

Preferred compounds of Formula II have the structures II-A, II-B, II-C and II-D which are set forth below in the section entitled Formulae. These compounds are available from the following suppliers:

10	Compound	Catalog Number	Supplier
	II-A	S5,479-9	Aldrich, Milwaukee, WI
	II-B	S5,755-0	Aldrich, Milwaukee, WI
	II-C	S5,740-2	Aldrich, Milwaukee, WI
	II-D	S5,751-8	Aldrich, Milwaukee, WI

- 15 In some embodiments, the pharmaceutical compositions of the present invention comprise compounds having Formula III which is set forth in the section below entitled Formulae. In compound of Formula III, R_1 is H or diethylamino; R_2 is -O- or - $N(C_6H_5)-$, and R_3 is -Br, Cl, or F.

- Preferred compounds of Formula III have the structure III-A and III-B which are set forth below in the section entitled Formulae.

These compounds are available from the following suppliers:

Compound	Catalog Number	Supplier
III-A	F21,855-5	Aldrich, Milwaukee, WI
III-B	C-390	Biosynth, Naperville, IL

25 Example 2

Pharmaceutical compositions are prepared using compounds of Formulas I, II and III which are commercially available from chemical suppliers such as Sigma, Aldrich, ICN, Ryan Scientific, George Uhe (Paramus, NJ), and Biosynth (Naperville, IL).

The pharmaceutical compositions are useful to treat individuals suffering from TNF-mediated diseases, disorders and conditions. Examples of TNF-mediated diseases, disorders and conditions include, for example, inflammatory diseases and autoimmune diseases such as rheumatoid arthritis (RA), multiple sclerosis (MS), Sjogren's syndrome, sarcoidosis, insulin dependent diabetes mellitus (IDDM), autoimmune thyroiditis, reactive arthritis, ankylosing spondylitis, scleroderma, polymyositis, dermatomyositis, psoriasis, vasculitis, Wegener's granulomatosis, Crohn's disease, ulcerative colitis, Lupus (SLE), Grave's disease, myasthenia gravis, autoimmune hemolytic anemia, autoimmune thrombocytopenia, asthma, cryoglobulinemia, primary biliary sclerosis and pernicious anemia. According to the present invention, individuals suffering from such diseases, disorders and conditions may be treated by administering to them a therapeutically effective amount of a pharmaceutical composition that comprises a compound having Formula I, II and III.

The method may include administration of compounds to mammals, preferably humans, in therapeutically effective amounts which are effective to inhibit TNF-mediated diseases. The dosage administered in any particular instance will depend upon factors such as the pharmacodynamic characteristics of the compound, its mode and route of administration; age, health, and weight of the recipient; nature and extent of symptoms; kind of concurrent treatment, frequency of treatment, and the effect desired.

It is contemplated that the daily dosage of a compound used in the method that is the invention will be in the range of from about 1 μ g to about 10 grams per day. In some preferred embodiments, the daily dosage compound will be in the range of from about 10 mg to about 1 gram per day. In some preferred embodiments, the daily dosage compound will be in the range of from about 100 mg to about 500 mg per day. It is contemplated that the daily dosage of a compound used in the method that is the invention will be in the range of from about 1 μ g to about 100 mg per kg of body weight, in some embodiments, from about 1 μ g to about 40 mg per kg body weight; in some embodiments from about 10 μ g to about 20 mg per kg per day, and in some embodiments 10 μ g to about 1 mg per kg per day.

Pharmaceutical compositions may be administered in a single dosage, divided dosages or in sustained release. In some preferred embodiments, the compound

- 24 -

will be administered in multiple doses per day. In some preferred embodiments, the compound will be administered in 3-4 doses per day.

Persons of ordinary skill will be able to determine dosage forms and amounts with only routine experimentation based upon the considerations of this invention.

The method of administering compounds include administration as a pharmaceutical composition orally in solid dosage forms, such as capsules, tablets, and powders, or in liquid dosage forms, such as elixirs, syrups, and suspensions. The compounds may also be administered parenterally in sterile liquid dosage forms or topically in a carrier. The compounds may be formulated into dosage forms according to standard practices in the field of pharmaceutical preparations. See *Remington's Pharmaceutical Sciences*, 18th Edition, A.R. Gennaro, Ed., Mack Publishing Company, Easton, Pennsylvania, which is incorporated herein by reference.

Compounds may be mixed with powdered carriers, such as lactose, sucrose, mannitol, starch, cellulose derivatives, magnesium stearate, and stearic acid for insertion into gelatin capsules, or for forming into tablets. Both tablets and capsules may be manufactured as sustained release products for continuous release of medication over a period of hours.

Liquid dosage forms for oral administration may contain coloring and flavoring to increase patient acceptance, in addition to a pharmaceutically acceptable diluent such as water, buffer or saline solution.

For parenteral administration, a compound may be mixed with a suitable carrier or diluent such as water, a oil, saline solution, aqueous dextrose (glucose), and related sugar solutions, and glycols such as propylene glycol or polyethylene glycols.

Solutions for parenteral administration contain preferably a water soluble salt of the compound. Stabilizing agents, antioxidizing agents and preservatives may also be added. Suitable antioxidizing agents include sodium bisulfite, sodium sulfite, and ascorbic acid, citric acid and its salts, and sodium EDTA. Suitable preservatives include benzalkonium chloride, methyl- or propyl-paraben, and chlorbutanol.

30 Example 3

- 25 -

In one embodiment of the invention, CIAM technology is used to identify compounds that inhibit interactions between CD4, and MHC/antigen/TCR complexes.

By inhibiting CD4, the T cell activation associated with MHC/antigen/TCR complexes can be reduced and immune responses suppressed accordingly. The crystal
5 structure of the CD4 complex shows distinct binding sites.

Peptidomimetics were developed and tested from surface loops of CD4. A peptidomimetic engineered from a CD4 domain inhibited T cell activation associated with MHC/antigen/TCR complexes.

Using the location of the functionally active amino acid sequence identified
10 using the peptidomimetic as the location of a functionally critical active site, the surface of the CD4 molecule was reviewed and a large cleft was identified that could be utilized for docking a pseudo-allosteric inhibitor. The interior of the cavity was mapped and a chemical database was searched. A compound with binding energy of -34Kcal/mol and having Formula IV was identified. This compound which is commercially available from
15 Salor/Aldrich (Catalog #S69, 246-8) showed activity in *in vitro* T cell activation assays.

Pharmaceutical compositions are prepared using the compound of Formula IV, V or VI which are set forth in the section below entitled Formulae. The pharmaceutical compositions are useful to treat individuals suffering from CD4-mediated diseases, disorders and conditions. Examples of CD4-mediated diseases, disorders and
20 conditions include, for example, inflammatory diseases and autoimmune diseases such as rheumatoid arthritis (RA), multiple sclerosis (MS), Sjogren's syndrome, sarcoidosis, insulin dependent diabetes mellitus (IDDM), autoimmune thyroiditis, reactive arthritis, ankylosing spondylitis, scleroderma, polymyositis, dermatomyositis, psoriasis, vasculitis, Wegener's granulomatosis, Crohn's disease, ulcerative colitis, Lupus (SLE), Grave's
25 disease, myasthenia gravis, autoimmune hemolytic anemia, autoimmune thrombocytopenia, asthma, cryoglobulinemia, primary biliary sclerosis and pernicious anemia. According to the present invention, individuals suffering from such diseases, disorders and conditions may be treated by administering to them a therapeutically effective amount of a pharmaceutical composition that comprises a compound having
30 either Formula IV, V or VI.

The method may include administration of compounds to mammals, preferably humans, in therapeutically effective amounts which are effective to inhibit CD4-mediated diseases. The dosage administered in any particular instance will depend upon factors such as the pharmacodynamic characteristics of the compound, its mode and
5 route of administration; age, health, and weight of the recipient, nature and extent of symptoms; kind of concurrent treatment, frequency of treatment, and the effect desired.

It is contemplated that the daily dosage of a compound used in the method that is the invention will be in the range of from about 1 μ g to about 10 grams per day. In some preferred embodiments, the daily dosage compound will be in the range of from
10 about 10 mg to about 1 gram per day. In some preferred embodiments, the daily dosage compound will be in the range of from about 100 mg to about 500 mg per day. It is contemplated that the daily dosage of a compound used in the method that is the invention will be in the range of from about 1 μ g to about 100 mg per kg of body weight, in some embodiments, from about 1 μ g to about 40 mg per kg body weight; in some embodiments
15 from about 10 μ g to about 20 mg per kg per day, and in some embodiments 10 μ g to about 1 mg per kg per day.

Pharmaceutical compositions may be administered in a single dosage, divided dosages or in sustained release. In some preferred embodiments, the compound will be administered in multiple doses per day. In some preferred embodiments, the
20 compound will be administered in 3-4 doses per day.

Persons of ordinary skill will be able to determine dosage forms and amounts with only routine experimentation based upon the considerations of this invention.

The method of administering compounds include administration as a
25 pharmaceutical composition orally in solid dosage forms, such as capsules, tablets, and powders, or in liquid dosage forms, such as elixirs, syrups, and suspensions. The compounds may also be administered parenterally in sterile liquid dosage forms or topically in a carrier. The compounds may be formulated into dosage forms according to standard practices in the field of pharmaceutical preparations. See *Remington's*
30 *Pharmaceutical Sciences*, 18th Edition, A.R. Gennaro, Ed., Mack Publishing Company, Easton, Pennsylvania, which is incorporated herein by reference.

Compounds may be mixed with powdered carriers, such as lactose, sucrose, mannitol, starch, cellulose derivatives, magnesium stearate, and stearic acid for insertion into gelatin capsules, or for forming into tablets. Both tablets and capsules may be manufactured as sustained release products for continuous release of medication over a 5 period of hours.

Liquid dosage forms for oral administration may contain coloring and flavoring to increase patient acceptance, in addition to a pharmaceutically acceptable diluent such as water, buffer or saline solution.

For parenteral administration, a compound may be mixed with a suitable 10 carrier or diluent such as water, a oil, saline solution, aqueous dextrose (glucose), and related sugar solutions, and glycols such as propylene glycol or polyethylene glycols. Solutions for parenteral administration contain preferably a water soluble salt of the compound. Stabilizing agents, antioxidizing agents and preservatives may also be added. Suitable antioxidizing agents include sodium bisulfite, sodium sulfite, and ascorbic acid, 15 citric acid and its salts, and sodium EDTA. Suitable preservatives include benzalkonium chloride, methyl- or propyl-paraben, and chlorbutanol.

Example 4

In one embodiment of the invention, CIAM technology is used to identify compounds that inhibit β -lactamase. Inhibition of the enzyme β -lactamase is useful to 20 render penicillin-resistant strains of bacteria, penicillin-sensitive. A cavity with the criteria described above proximal from the active site of β -lactamase was identified by β -factors and thermodynamic analysis.

The surface of the β -lactamase molecule was reviewed and a proximal suitable cleft was identified that could be utilized for docking an allosteric inhibitor. The 25 cavity was mapped and a chemical database was searched. A series of compounds were identified. These compounds are shown as Formulae VII-XIX in the section below entitled Formulae. They are commercially available from several suppliers including Aldrich (Milwaukee, WI; www.sigma-aldrich.com), Sigma (www.sigma-aldrich.com), Fluka (www.sigma-aldrich.com), ICN (www.icnpharm.com), Ryan Scientific (Isle of 30 Palms, SC), SynTec (Germany) and Bayer (Leverkusen, Germany; www.bayer.com).

- Pharmaceutical compositions are prepared using one of the compounds selected from the group of Formula VII-XIX. The pharmaceutical compositions are useful to treat individuals suffering from bacterial infectious, particularly those which are penicillin resistant. According to the present invention, individuals suffering from such
- 5 infections may be treated by administering to them a therapeutically effective amount of a pharmaceutical composition that comprises a compound having Formula VII, VIII, IX, X, XI, XII, XIII, XIV, XV, XVI, XVII, XVIII or XIX in combination with penicillin-derived antibiotic.

- The method may include administration of compounds to mammals,
- 10 preferably humans, in therapeutically effective amounts which are effective to inhibit β -lactamase in order to render penicillin-resistant strains of bacteria penicillin-sensitive. The dosage administered in any particular instance will depend upon factors such as the pharmacodynamic characteristics of the compound, its mode and route of administration, age, health, and weight of the recipient; nature and extent of symptoms; kind of
- 15 concurrent treatment, frequency of treatment, and the effect desired.

- It is contemplated that the daily dosage of the compound used in the method that is the invention will be in the range of from about 1 μ g to about 10 grams per day. In some preferred embodiments, the daily dosage compound will be in the range of from about 10 mg to about 1 gram per day. In some preferred embodiments, the daily
- 20 dosage compound will be in the range of from about 100 mg to about 500 mg per day. It is contemplated that the daily dosage of a compound used in the method that is the invention will be in the range of from about 1 μ g to about 100 mg per kg of body weight, in some embodiments, from about 1 μ g to about 40 mg per kg body weight, in some embodiments from about 10 μ g to about 20 mg per kg per day, and in some embodiments
- 25 10 μ g to about 1 mg per kg per day.

Pharmaceutical compositions may be administered in a single dosage, divided dosages or in sustained release. In some preferred embodiments, the compound will be administered in multiple doses per day. In some preferred embodiments, the compound will be administered in 3-4 doses per day.

Persons of ordinary skill will be able to determine dosage forms and amounts with only routine experimentation based upon the considerations of this invention.

- The method of administering compounds include administration as a
- 5 pharmaceutical composition orally in solid dosage forms, such as capsules, tablets, and powders, or in liquid dosage forms, such as elixirs, syrups, and suspensions. The compounds may also be administered parenterally in sterile liquid dosage forms or topically in a carrier. The compounds may be formulated into dosage forms according to standard practices in the field of pharmaceutical preparations. See *Remington's*
- 10 *Pharmaceutical Sciences*, 18th Edition, A.R. Gennaro, Ed., Mack Publishing Company, Easton, Pennsylvania, which is incorporated herein by reference.

- Compounds may be mixed with powdered carriers, such as lactose, sucrose, mannitol, starch, cellulose derivatives, magnesium stearate, and stearic acid for insertion into gelatin capsules, or for forming into tablets. Both tablets and capsules may be
- 15 manufactured as sustained release products for continuous release of medication over a period of hours.

- Liquid dosage forms for oral administration may contain coloring and flavoring to increase patient acceptance, in addition to a pharmaceutically acceptable diluent such as water, buffer or saline solution.
- 20 For parenteral administration, a compound may be mixed with a suitable carrier or diluent such as water, a oil, saline solution, aqueous dextrose (glucose), and related sugar solutions, and glycols such as propylene glycol or polyethylene glycols. Solutions for parenteral administration contain preferably a water soluble salt of the compound. Stabilizing agents, antioxidizing agents and preservatives may also be added.
- 25 Suitable antioxidizing agents include sodium bisulfite, sodium sulfite, and ascorbic acid, citric acid and its salts, and sodium EDTA. Suitable preservatives include benzalkonium chloride, methyl- or propyl-paraben, and chlorbutanol.

- In some embodiments, the pharmaceutical compositions of the present invention used in the methods of the present invention comprise compounds having
- 30 Formulae VII - XVI.

In some embodiments, the pharmaceutical compositions of the present invention comprise compounds having Formula VII.

In some embodiments, the pharmaceutical compositions of the present invention comprise compounds having Formula VIII.

- 5 Compounds according to Formula VIII may have at position R_1 a group having Formula 8-1-1, 8-1-2, 8-1-3, 8-1-4, 8-1-5, 8-1-6, 8-1-7, 8-1-8, 8-1-9 and 8-1-10 which are set forth below in the section entitled Formulae.

- Compounds according to Formula VIII may have at position R_2 and R_2 are, independently, -H, - C_1 , - C_2 , - C_3 straight or branched, - C_4 straight or branched, - C_5 straight or branched, C_6 straight or branched, - C_7 straight or branched, and - C_8 straight or branched. R_2 and R_2 are preferably identical to each other. R_2 and R_2 are preferably -H or the - C_8 branched 4-tert-octyl.
- 10

Some preferred compounds of Formula VIII include compounds wherein:

- R_1 is 8-1-1, R_2 is -H, and R_3 is -H;
- 15 R_1 is 8-1-1, R_2 is 4-tert-octyl, and R_3 is 4-tert-octyl;
- R_1 is 8-1-2, R_2 is -H and R_3 is -H;
- R_1 is 8-1-3, R_2 is -H and R_3 is -H;
- R_1 is 8-1-4, R_2 is -H and R_3 is -H;
- R_1 is 8-1-5, R_2 is -H and R_3 is -H;
- 20 R_1 is 8-1-6, R_2 is -H and R_3 is -H;
- R_1 is 8-1-7, R_2 is -H and R_3 is -H;
- R_1 is 8-1-8, R_2 is -H and R_3 is -H;
- R_1 is 8-1-9, R_2 is -H and R_3 is -H; and
- R_1 is 8-1-10, R_2 is -H and R_3 is -H.

- 25 Some preferred compounds of Formula VIII include structure VIII-A, VIII-B, VIII-C, VIII-D, VIII-E, VIII-F, VIII-G, VIII-H, VIII-I, VIII-J or VIII-K which are set forth below in the section entitled Formulae.

Compound	Source	Catalog Number
VIII-A.	ALDRICH	F28,168-9
30 VIII-B.	ALDRICH	25,762-1
VIII-C.	SIGMA-ALDRICH	S68,073-7

VIII-D.	SIGMA-ALDRICH	S15,490-3
VIII-E.	SIGMA-ALDRICH	S15,495-4
VIII-F.	SIGMA-ALDRICH	S15,498-9
VIII-G.	SIGMA-ALDRICH	S15,504-0
5 VIII-H.	SIGMA-ALDRICH	S15,505-5
VIII-I.	SIGMA-ALDRICH	R17,712-1
VIII-J.	SIGMA-ALDRICH	R17,271-5
VIII-K.	SIGMA-ALDRICH	R17,703-2

- 10 In some embodiments, the pharmaceutical compositions of the present invention comprise compounds having Formula IX.

- Compounds according to Formula IX may have at position R_1 , R_2 , R_3 and R_4 , independently, -H, $-OC_6H_5Cl$, $-N(CH_3)_2$, $-OCH_3$, $-CH_3$, $-OH$ or $-halogen$, and if $halogen$, preferably $-Cl$. In some preferred embodiments, R_1 is $-OCH_3$, $-OH$ or $-halogen$,
 15 and if $halogen$, preferably $-Cl$. In some preferred embodiments R_2 is $-H$, $-N(CH_3)_2$, $-OCH_3$, $-CH_3$, or $-halogen$, and if $halogen$, preferably $-Cl$. In some preferred embodiments R_3 is $-OCH_3$, $-CH_3$, $-OH$ or $-halogen$, and if $halogen$, preferably $-Cl$. In some preferred embodiments R_4 is $-OC_6H_5Cl$, $-OCH_3$, $-CH_3$ or $-OH$.

Some preferred compounds of Formula IX include compounds wherein:

- 20 R_1 is $-Cl$, R_2 is $-H$, R_3 is $-Cl$ and R_4 is OC_6H_5Cl ;
 R_1 is $-N(CH_3)_2$, R_2 is $-H$, R_3 is $-Cl$ and R_4 is $-OCH_3$;
 R_1 is $-OCH_3$, R_2 is $-H$, R_3 is $-OCH_3$ and R_4 is $-OCH_3$;
 R_1 is $-Cl$, R_2 is $-H$, R_3 is $-Cl$ and R_4 is $-OCH_3$;
 R_1 is $-Cl$, R_2 is $-H$, R_3 is $-CH_3$ and R_4 is $-OCH_3$;
 25 R_1 is $-OCH_3$, R_2 is $-Cl$, R_3 is $-H$ and R_4 is $-OH$;
 R_1 is $-OH$, R_2 is $-H$, R_3 is $-Cl$ and R_4 is $-OCH_3$;
 R_1 is $-OCH_3$, R_2 is $-H$, R_3 is $-CH_3$ and R_4 is $-OCH_3$;
 R_1 is $-Cl$, R_2 is $-Cl$, R_3 is $-OCH_3$ and R_4 is $-OCH_3$;
 R_1 is $-Cl$, R_2 is $-H$, R_3 is $-OCH_3$ and R_4 is $-OCH_3$;
 30 R_1 is $-OCH_3$, R_2 is $-H$, R_3 is $-OCH_3$ and R_4 is $-OCH_3$;
 R_1 is $-OCH_3$, R_2 is $-CH_3$, R_3 is $-Cl$ and R_4 is $-H$;

R₁ is -OH, R₂ is -H, R₃ is -Cl and R₄ is -CH₃; and

R₁ is -OCH₃, R₂ is -OCH₃, R₃ is -OH and R₄ is -H.

Some preferred compounds of Formula IX include structure IX-A, IX-B, IX-C, IX-D, IX-E, IX-F, IX-G, IX-H, IX-I, IX-J, IX-K, IX-L, IX-M or IX-N which are

5 set forth below in the section entitled Formulae.

Compound	Source	Catalog Number
IX-A.	SIGMA-ALDRICH	S50,872-1
IX-B.	SIGMA-ALDRICH	S69,044-9
IX-C.	SIGMA-ALDRICH	S69,613-7
10 IX-D.	SIGMA-ALDRICH	S69,516-5
IX-E.	SIGMA-ALDRICH	S12,931-3
IX-F.	SIGMA-ALDRICH	S72,315-0
IX-G.	SIGMA-ALDRICH	S69,055-4
IX-H.	SIGMA-ALDRICH	S76,872-3
15 IX-I.	SIGMA-ALDRICH	S90,369-8
IX-J.	SIGMA-ALDRICH	S90,370-1
IX-K.	SIGMA-ALDRICH	S74,299-6
IX-L.	SIGMA-ALDRICH	S72,956-6
IX-M.	SIGMA-ALDRICH	S91,728-1
20 IX-N.	SIGMA-ALDRICH	S91,730-3

In some embodiments, the pharmaceutical compositions of the present invention comprise compounds having Formula X.

Compounds according to Formula X may have at position R₁ a group having Formula 10-1-1, 10-1-2 or 10-1-3 which are set forth below in the section entitled

25 Formulae.

Compounds according to Formula X may have at position R₂, R₃ and R₄ are, independently, -H, -NO₂, -NH₂ or -CH₃. R₂ is preferably -H or -NH₂. R₃ is preferably -NO₂ or -NH₂. R₄ is preferably -H or -CH₃.

Some preferred compounds of Formula X include compounds wherein:

30

R₁ is 10-1-1, R₂ is -H, R₃ is -NO₂ and R₄ is -H,

R₁ is 10-1-2, R₂ is -H, R₃ is -NO₂ and R₄ is -H; and

R₁ is 10-1-3, R₂ is -NH₂, R₃ is -NH₂ and R₄ is -CH₃.

Some preferred compounds of Formula X include structure X-A, X-B or X-C which are set forth below in the section entitled Formulae.

5	Compound	Source	Catalog Number
	X-A.	RYAN SCIENTIFIC	NRB01150
	X-B.	SIGMA	S93,056-3
	X-C.	ALDRICH	21,222-9

In some embodiments, the pharmaceutical compositions of the present

- 10 invention comprise compounds having Formula XI.

Compounds according to Formula XI may have at position R₁ and R₂, independently, a group having Formula 11-1/2-1, 11-1/2-2, 11-1/2-3, 11-1/2-4, which are set forth below in the section entitled Formulae, or -H, -NO₂ or -OH.

Compounds according to Formula XI may have at position R₃ either -H, -

- 15 NH₂, -OH, or halogen, and when R₃ is halogen, it is preferably -Cl or -Br.

Compounds according to Formula XI may have at position R₃ either -H, -

NH₂, -OH, or halogen, and when R₃ is halogen, it is preferably -Cl or -Br.

Compounds according to Formula XI may have at position R₄ either -H or -

CH(CH₃)₃.

- 20 Some preferred compounds of Formula XI include compounds wherein:

R₁ is 11-1/2-1, R₂ is 11-1/2-1, R₃ is -H and R₄ is -H;

R₁ is 11-1/2-2, R₂ is 11-1/2-2, R₃ is -NH₂ and R₄ is -H;

R₁ is 11-1/2-3, R₂ is 11-1/2-3, R₃ is -Cl and R₄ is -H;

R₁ is 11-1/2-4, R₂ is -H, R₃ is -NO₂ and R₄ is -H; and

- 25 R₁ is -NO₂, R₂ is -OH, R₃ is -OH and R₄ is -H.

Some preferred compounds of Formula XI include structure XI-A, XI-B,

XI-C, XI-D or XI-E which are set forth below in the section entitled Formulae.

	Compound	Source	Catalog Number
	XI-A.	SIGMA-ALDRICH	S18,982-0
30	XI-B.	SIGMA-ALDRICH	S18,611-2
	XI-C.	SIGMA	S3,634-0

XI-D.	SIGMA	S86,927-9
XI-E.	SIGMA	S53,622-9

In some embodiments, the pharmaceutical compositions of the present invention comprise compounds having Formula XII.

- 5 Compounds according to Formula XII may have at position R₁ either N-pyridinium, 4-methyl-N-pyridinium, 4-dimethylamino-N-pyridinium, 3-methyl-N-pyridinium, N-pyridinium, 2,6 dimethyl-N-pyridinium, 3,5 dimethyl-N-pyridinium, 3-ethyl-N-pyridinium, 12-1-1, 12-1-2 which are set forth below in the section entitled Formulae, or 4-ethyl-N-pyridinium, 4-benzyl-N-pyridinium, N-quinolinyl or CH₃.

- 10 Some preferred compounds of Formula XII include compounds wherein:

R₁ is N-pyridinium and R₂ is NO₂;

R₁ is 4-methyl-N-pyridinium and R₂ is NO₂;

R₁ is 4-dimethylamino-N-pyridinium and R₂ is NO₂;

R₁ is 3-methyl-N-pyridinium and R₂ is NO₂;

- 15 R₁ is N-pyridinium and R₂ is NO₂;

R₁ is 2,6 dimethyl-N-pyridinium and R₂ is NO₂;

R₁ is 3,5 dimethyl-N-pyridinium and R₂ is NO₂;

R₁ is 3-ethyl-N-pyridinium and R₂ is NO₂;

R₁ is 12-1-1 and R₂ is NO₂;

- 20 R₁ is 12-1-2 and R₂ is NO₂;

R₁ is 4-ethyl-N-pyridinium and R₂ is NO₂;

R₁ is 4-benzyl-N-pyridinium and R₂ is NO₂;

R₁ is N-quinolinyl and R₂ is NO₂; and

R₁ is CH₃ and R₂ is H..

- 25 Some preferred compounds of Formula XII include structure XII-A, XII-B, XII-C, XII-D, XII-E, XII-F, XII-G, XII-H, XII-I, XII-J, XII-K, XII-L, XII-M or XII-N, which are set forth below in the section entitled Formulae.

Compound	Source	Catalog Number
XII-A.	SIGMA	S14,318-9
30 XII-B.	SIGMA	S96,676-2

	XII-C.	SIGMA	S14,440-1
	XII-D.	SIGMA	S96,664-9
	XII-E.	SIGMA	S96,668-1
	XII-F.	SIGMA	S96,670-3
5	XII-G.	SIGMA	S14,386-3
	XII-H.	SIGMA	S96,674-6
	XII-I.	SIGMA	S96,682-7
	XII-J.	SIGMA	S96,677-0
	XII-K.	SIGMA	S96,679-7
10	XII-L.	SIGMA	S96,685-1
	XII-M.	SIGMA	S14,675-7
	XII-N.	SIGMA-ALDRICH	S67,954-2

- In some embodiments, the pharmaceutical compositions of the present
- 15 invention comprise compounds having Formula XIII which is set forth in the section below entitled Formulae. Compounds having Formula XIII are available from SIGMA-ALDRICH, Catalog number S42,591-5.

In some embodiments, the pharmaceutical compositions of the present invention comprise compounds having Formula XIV.

- 20 Compounds according to Formula XIV may have at position R₁ either -OCH₃, -NO₂ or -halogen, and if -halogen, preferably -Cl.

Compounds according to Formula XIV may have at position R₂ either -H, -NO₂ or -halogen, and if -halogen, preferably -Cl.

- Some preferred compounds of Formula XIV include compounds wherein:
- 25 R₁ is -Cl and R₂ is -Cl;
 R₁ is -OCH₃ and R₂ is -H;
 R₁ is -Cl and R₂ is -H; and
 R₁ is -NO₂ and R₂ is NO₂.

- Some preferred compounds of Formula XIV include structure XIV-A,
- 30 XIV-B, XIV-C or XIV-D which are set forth in the section below entitled Formulae.

Compound	Source	Catalog Number
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- 36 -

XIV-A.	SIGMA	S6,886-2
XIV-B.	SIGMA	S12,703-5
XIV-C.	SIGMA	S62,321-0
XIV-D.	SIGMA	S24,232-2

5 In some embodiments, the pharmaceutical compositions of the present invention comprise compounds having Formula XV.

Compounds according to Formula XV may have at position R₁ either -H, -NO₂, -FSO₂, -CH₃, -OCH₃, -SO₂CH₃, 15-1-1, 15-1-2 or 15-1-3 which are set forth in the section below entitled Formulae.

10 Compounds according to Formula XV may have at position R₂ either -H, -OH, or -NO₂.

Compounds according to Formula XV may have at position R₃ either -H or -OH.

Compounds according to Formula XV may have at position R₄ either -H, 15 15-4-1, 15-4-2, 15-4-3, 15-4-4, 15-4-5, 15-4-6, 15-4-7, 15-4-8, 15-4-9, 15-4-10, 15-4-11, 15-4-12 which are set forth in the section below entitled Formulae or halogen, and when R₄ is halogen, it is preferably -Cl.

Some preferred compounds of Formula XV include compounds wherein:

- 20 R₁ is -NO₂, R₂ is -H, R₃ is -OH and R₄ is -Cl;
 R₁ is -H, R₂ is -OH, R₃ is -H and R₄ is -15-4-1;
 R₁ is -FSO₂, R₂ is -NO₂, R₃ is -OH, and R₄ is -H;
 R₁ is 15-1-1, R₂ is -NO₂, R₃ is -OH and R₄ is -H;
 R₁ is -H, R₂ is -H, R₃ is -H and R₄ is 15-4-2;
 R₁ is -H, R₂ is -H, R₃ is -OH and R₄ is 15-4-3;
 25 R₁ is -H, R₂ is -H, R₃ is -OH and R₄ is 15-4-5;
 R₁ is -H, R₂ is -OH, R₃ is -OCH₃ and R₄ is 15-4-6;
 R₁ is -OCH₃, R₂ is -H, R₃ is -OH and R₄ is 15-4-7;
 R₁ is -H, R₂ is -H, R₃ is -OH and R₄ is 15-4-8;
 R₁ is -H, R₂ is -H, R₃ is -OH and R₄ is 15-4-9;
 30 R₁ is -OCH₃, R₂ is -H, R₃ is -H and R₄ is 15-4-10;

R_1 is $-FSO_2$, R_2 is $-H$, R_3 is $-OH$ and R_4 is 15-4-11;

R_1 is $-H$, R_2 is $-H$, R_3 is $-H$ and R_4 is 15-4-12;

R_1 is $-SO_2CH_3$, R_2 is $-H$, R_3 is $-OH$ and R_4 is $-H$;

R_1 is 15-1-2, R_2 is $-H$, R_3 is $-OH$ and R_4 is $-H$; and

5 R_1 is 15-1-3, R_2 is $-NO_2$, R_3 is $-OH$ and R_4 is $-H$.

Some preferred compounds of Formula XV include structure XV-A, XV-B, XV-C, XV-D, XV-E, XV-F, XV-G, XV-H, XV-I, XV-J, XV-K, XV-L, XV-M, XV-N, XV-O, XV-P, XV-Q or XV-R which are set forth in the section below entitled Formulae.

	Compound	Source	Catalog Number
10	XV-A.	SIGMA	S72,767-9
	XV-B.	SIGMA	S72,772-5
	XV-C.	SIGMA	S73,689-9
	XV-D.	BAYER CORP.	25/08
	XV-E.	SIGMA	S50,245-6
15	XV-F.	SIGMA	S65,507-4
	XV-G.	SIGMA	S78,072-3
	XV-H.	SIGMA	S79,426-0
	XV-I.	SIGMA	S79,453-8
	XV-J.	SIGMA	S80,012-0
20	XV-K.	SIGMA	S84,486-1
	XV-L.	RYAN	NRB01429
	XV-M.	SIGMA	S92,407-5
	XV-N.	SIGMA	S72,781-4

In some embodiments, the pharmaceutical compositions of the present

25 invention comprise compounds having Formula XVI.

Compounds according to Formula XVI may have at position R_1 either $-H$, $-SO_3$ or halogen, and when R_4 is halogen, it is preferably $-Cl$.

Compounds according to Formula XVI may have at position R_2 either $-H$ or $-CH_3$.

Compounds according to Formula XVI may have at position R_3 either -H, -OCH₃, -NSO₂, -NO₂, 16-3-1 which is set forth below in the section entitled Formulae, or halogen, and when R_3 is halogen, it is preferably -F.

- Compounds according to Formula XVI may have at position R_4 either -H, -OCH₃, -SO₃ or halogen, and when R_4 is halogen, it is preferably -Cl.

Compounds according to Formula XVI may have at position R_5 either -H, -NO₂ or halogen, and when R_5 is halogen, it is preferably -Cl.

Compounds according to Formula XVI may have at position R_6 either -H, -C(CH₃)₂ or phenyl.

- 10 Some preferred compounds of Formula XVI include compounds wherein:

R_1 is -H, R_2 is -CH₃, R_3 is -OCH₃, R_4 is -OCH₃, R_5 is -H and R_6 is -

H,

R_1 is -H, R_2 is -CH₃, R_3 is -NSO₂, R_4 is -H, R_5 is -H and R_6 is -H,

R_1 is -H, R_2 is -CH₃, R_3 is -OCH₃, R_4 is -Cl, R_5 is -H and R_6 is -H,

- 15 R_1 is -NSO₂, R_2 is -CH₃, R_3 is -OCH₃, R_4 is -SO₃, R_5 is -NO₂ and R_6 is

-H,

R_1 is -H, R_2 is -H, R_3 is -H, R_4 is -H, R_5 is -H and R_6 is -H,

R_1 is -H, R_2 is -H, R_3 is -Cl, R_4 is -Cl, R_5 is -H and R_6 is -H,

R_1 is -H, R_2 is -CH₃, R_3 is -F, R_4 is -H, R_5 is -H and R_6 is -H,

- 20 R_1 is -H, R_2 is -CH₃, R_3 is -16-3-1, R_4 is -H, R_5 is -H and R_6 is -H,

R_1 is -H, R_2 is -CH₃, R_3 is -H, R_4 is -H, R_5 is -H and R_6 is -C(CH₃)₂.

R_1 is -H, R_2 is -CH₃, R_3 is -OCH₃, R_4 is -OCH₃, R_5 is -Cl and R_6 is -

H,

R_1 is -H, R_2 is -CH₃, R_3 is -F, R_4 is -H, R_5 is -H and R_6 is -H,

- 25 R_1 is -Cl, R_2 is -H, R_3 is -H, R_4 is -H, R_5 is -H and R_6 is -H,

R_1 is -Cl, R_2 is -H, R_3 is -OCH₃, R_4 is -H, R_5 is -H and R_6 is -H, or

R_1 is -H, R_2 is -CH₃, R_3 is -H, R_4 is -H, R_5 is -H and R_6 is phenyl.

- Some preferred compounds of Formula XVI include structure XVI-A, XVI-B, XVI-C, XVI-D, XVI-E, XVI-F, XVI-G, XVI-H, XVI-I, XVI-J, XVI-K, XVI-L, XVI-M or XVI-N which are set forth below in the section entitled Formulae.

Compound	Source	Catalog Number
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- 39 -

	XVI-A.	SIGMA	S53,065-4
	XVI-B.	SYNTEC, GERMANY	ST 58/4
	XVI-C.	SIGMA	S62,937-5
	XVI-D.	SIGMA	S50,191-3
5	XVI-E.	SIGMA	S21,210-5
	XVI-F.	SIGMA	S21,212-1
	XVI-G.	SIGMA	S6,965-6
	XVI-H.	SIGMA	S6,971-0
	XVI-I.	SIGMA	S7,002-6
10	XVI-J.	SIGMA	S21,225-3
	XVI-K.	SIGMA	S21,234-2
	XVI-L.	SIGMA	S21,241-5
	XVI-M.	SIGMA	S21,243-1
	XVI-N.	SIGMA	S63,263-5
15	XVI-O.	SIGMA	S21,212-1
	XVI-P.	SIGMA	S38,916-1
	XVI-Q.	SIGMA	S50,242-0
	XVI-R.	SIGMA	S62,979-0

In some embodiments, the pharmaceutical compositions of the present
20 invention comprise compounds having Formula XVII (SIGMA S86,927-9).

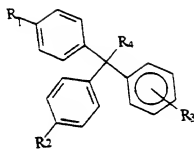
In some embodiments, the pharmaceutical compositions of the present
invention comprise compounds having Formula XVIII (RYAN SCIENTIFIC E191B)

In some embodiments, the pharmaceutical compositions of the present
invention comprise compounds having Formula XIX (SIGMA S12,962-3).

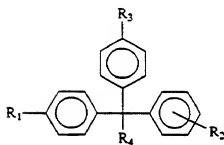
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FORMULAE

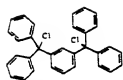


FORMULA I

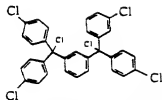


FORMULA II

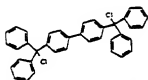
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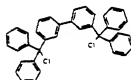
FORMULA II-A



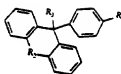
FORMULA II-B



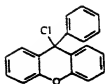
FORMULA II-C



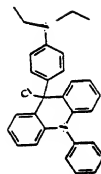
FORMULA II-D



FORMULA III



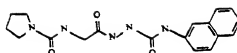
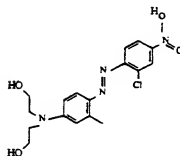
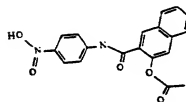
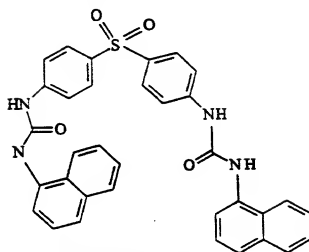
FORMULA III-A



FORMULA III-B

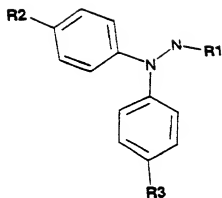
06720647-071701

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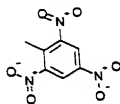
**FORMULA IV****FORMULA V****FORMULA VI****FORMULA VII**

09720647-071703

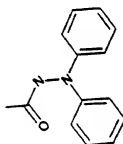
- 43 -



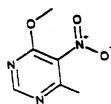
FORMULA VIII



FORMULA 8-1-1



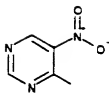
FORMULA 8-1-2



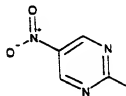
FORMULA 8-1-3

102720-2490240

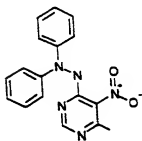
- 44 -



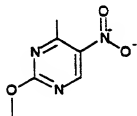
FORMULA 8-1-4



FORMULA 8-1-5

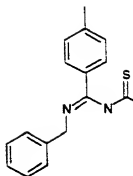


FORMULA 8-1-6

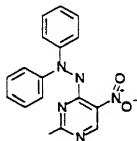


FORMULA 8-1-7

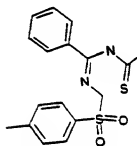
- 45 -



FORMULA 8-1-8



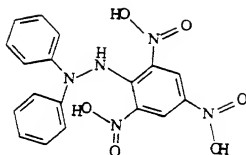
FORMULA 8-1-9



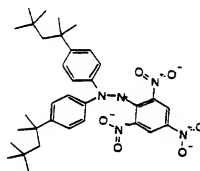
FORMULA 8-1-10

06720647-07333

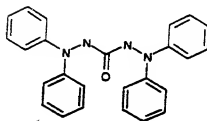
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FORMULA 8-A

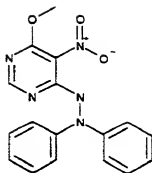
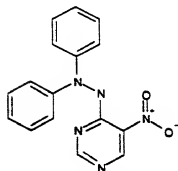
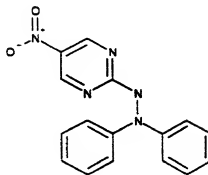


FORMULA 8-B

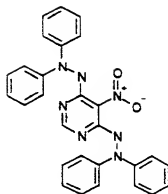


FORMULA 8-C

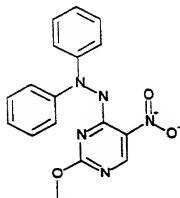
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**FORMULA 8-D****FORMULA 8-E****FORMULA 8-F**

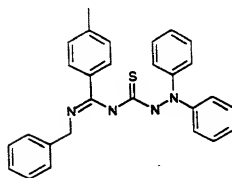
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FORMULA 8-G



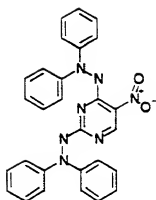
FORMULA 8-H



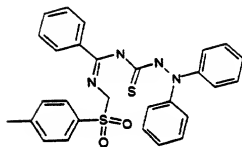
FORMULA 8-I

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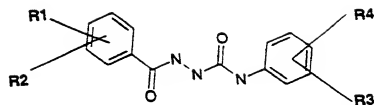
FORMULA 8-J



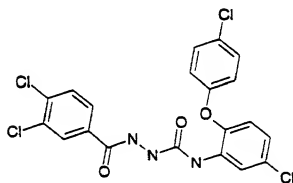
FORMULA 8-K

06722647-003700
302220-240220

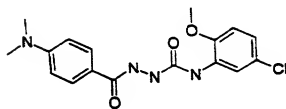
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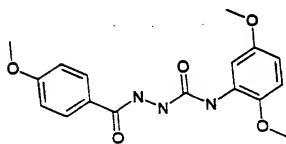
FORMULA IX



FORMULA IX-A

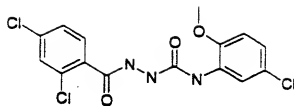


FORMULA IX-B

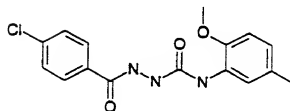


FORMULA IX-C

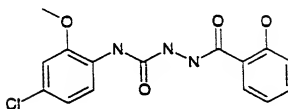
- 51 -



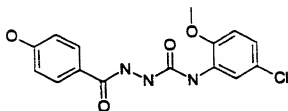
FORMULA IX-D



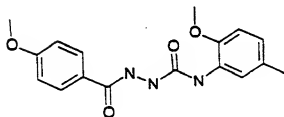
FORMULA IX-E



FORMULA IX-F

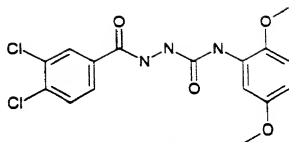


FORMULA IX-G

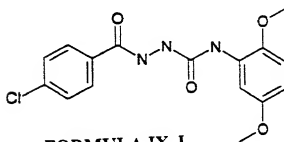


FORMULA IX-H

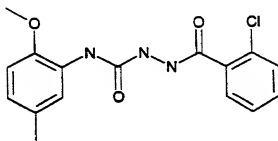
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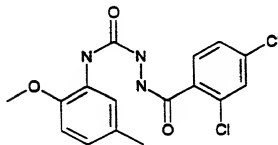
FORMULA IX-I



FORMULA IX-J

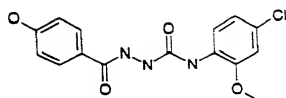


FORMULA IX-K

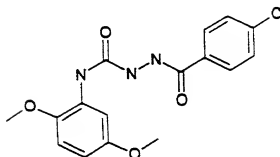


FORMULA IX-L

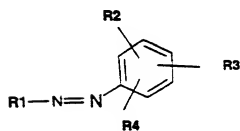
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FORMULA IX-M



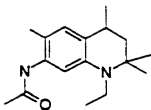
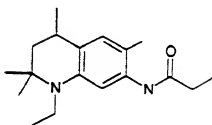
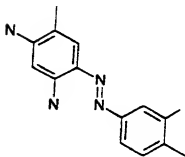
FORMULA IX-N



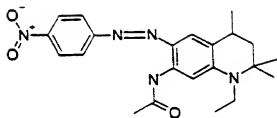
FORMULA X

09720647-023704

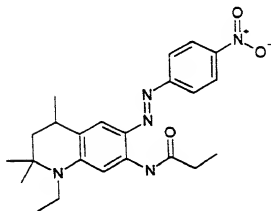
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**FORMULA 10-1-1****FORMULA 10-1-2****FORMULA 10-1-3**0072047-07344
102740-2402240

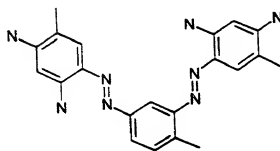
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FORMULA X-A



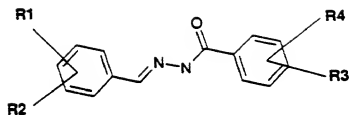
FORMULA X-B



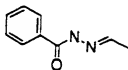
FORMULA X-C

0070647-01701

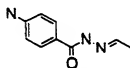
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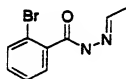
FORMULA XI



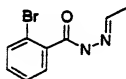
FORMULA 11-1/2-1



FORMULA 11-1/2-2

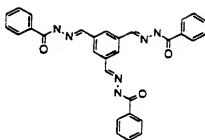


FORMULA 11-1/2-3



FORMULA 11-1/2-4

06720647-071704

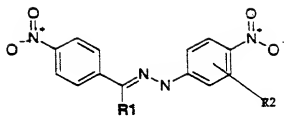


Chemical structure of compound 10: A central benzene ring substituted with a 4-nitrobenzoyl group, a 4-nitrobenzyl group, and a 4-nitrobenzylidene group.

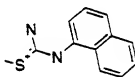
CC(C)(C)c1cc(OC(C)(C)C)c(CN=NC(=O)c2ccc(cc2)N=CN=C3C=CC=C3)cc1O=[N+]([O-])c1cc(C=NNC(=O)c2ccc(O)cc2)cc([N+](=O)[O-])cc1

FORMULA XI-E

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FORMULA XII

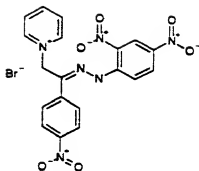


FORMULA 12-1-1

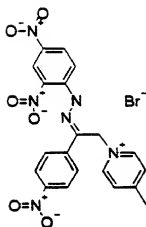


FORMULA 12-1-2

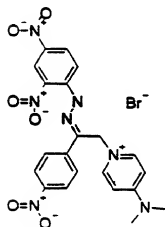
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FORMULA XII-A



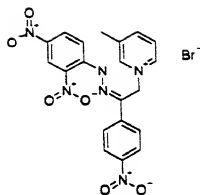
FORMULA XII-B



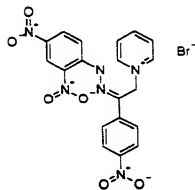
FORMULA XII-C

06720647-073701

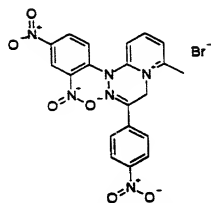
- 60 -



FORMULA XII-D

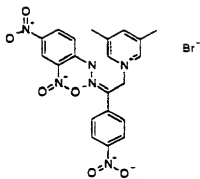


FORMULA XII-E

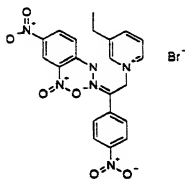


FORMULA XII-F

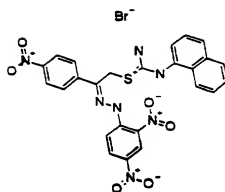
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FORMULA XII-G



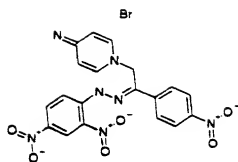
FORMULA XII-H



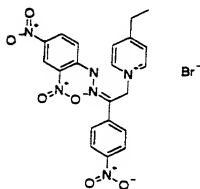
FORMULA XII-I

00720647-073701

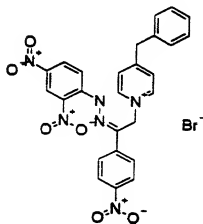
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FORMULA XII-J



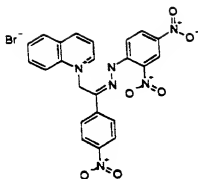
FORMULA XII-K



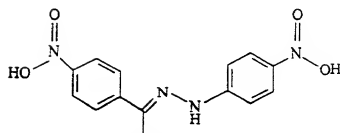
FORMULA XII-L

09/20047-071701

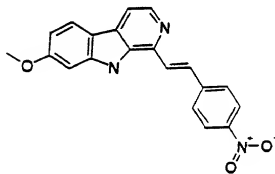
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FORMULA XII-M



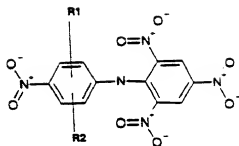
FORMULA XII-N



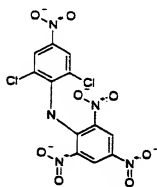
FORMULA XIII

00226647-023701

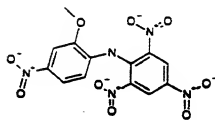
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FORMULA XIV



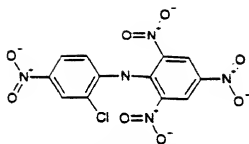
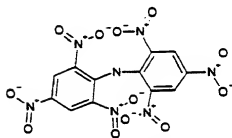
FORMULA XIV-A



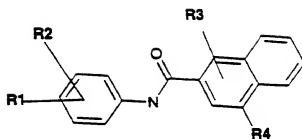
FORMULA XIV-B

09720647-021701

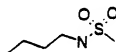
- 65 -

**FORMULA XIV-C****FORMULA XIV-D**

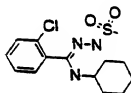
- 66 -



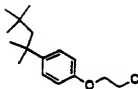
FORMULA XV



FORMULA 15-1-1

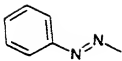
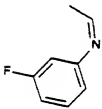


FORMULA 15-1-2

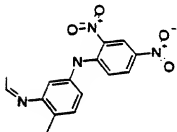


FORMULA 15-1-3

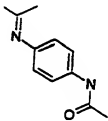
00720647-01701

**FORMULA 15-4-1**

FORMULA 15-4-2



FORMULA 15-4-3

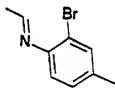


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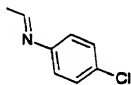
- 68 -



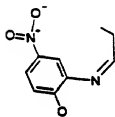
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FORMULA 15-4-6

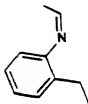


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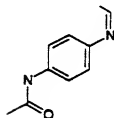


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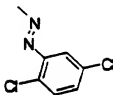
- 69 -



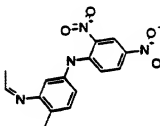
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FORMULA 15-4-10

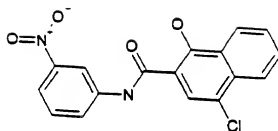


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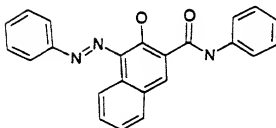


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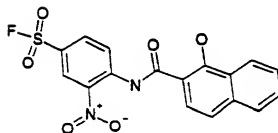
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FORMULA XV-A



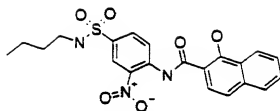
FORMULA XV-B



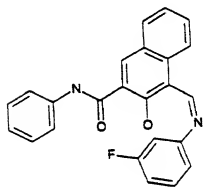
FORMULA XV-C

06720647-023701

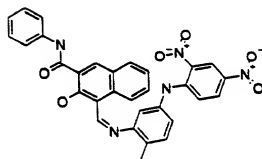
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FORMULA XV-D

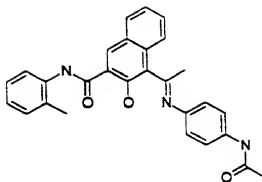


FORMULA XV-E

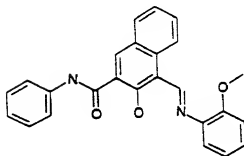


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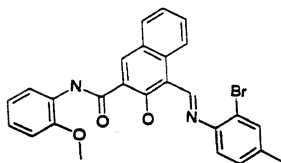
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FORMULA XV-G

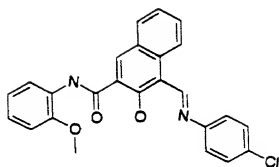


FORMULA XV-H

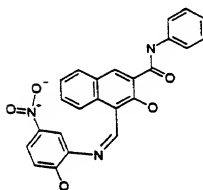


FORMULA XV-I

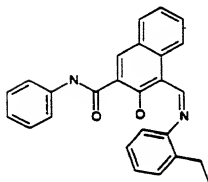
- 73 -



FORMULA XV-J



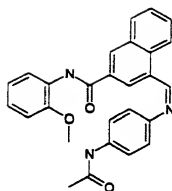
FORMULA XV-K



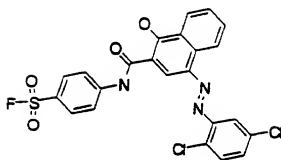
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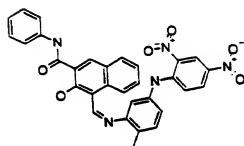
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FORMULA XV-M



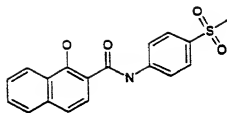
FORMULA XV-N



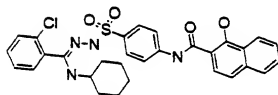
FORMULA XV-O

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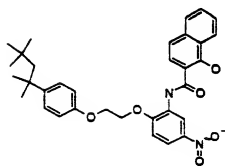
- 75 -



FORMULA XV-P

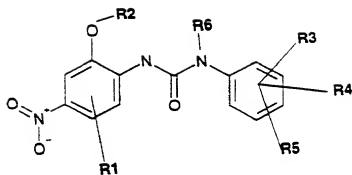


FORMULA XV-Q



FORMULA XV-R

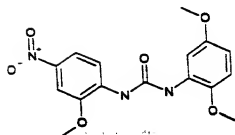
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FORMULA XVI

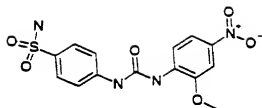


FORMULA 16-3-1

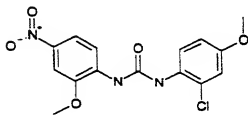


FORMULA XVI-A

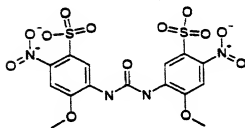
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FORMULA XVI-B



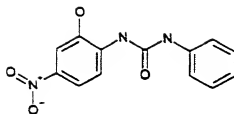
FORMULA XVI-C



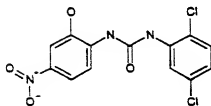
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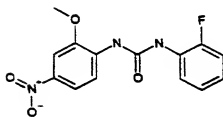
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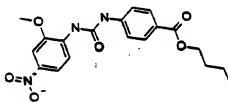
FORMULA XVI-E



FORMULA XVI-F



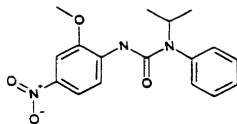
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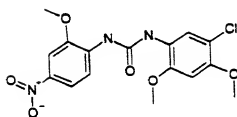
FORMULA XVI-H

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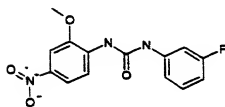
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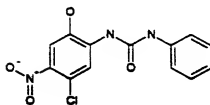
FORMULA XVI-I



FORMULA XVI-J



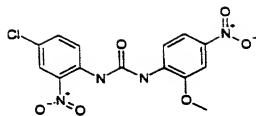
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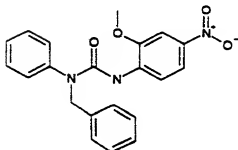
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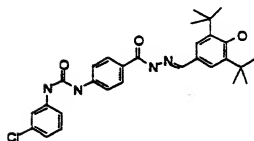
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FORMULA XVI-M

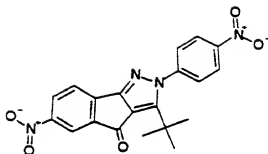


FORMULA XVI-N

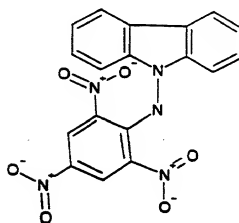


FORMULA XVII

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FORMULA XVIII



FORMULA XIX

0972047-07201

CLAIMS

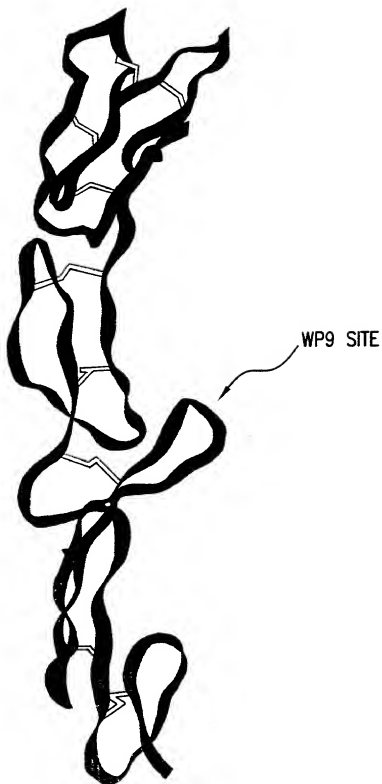
1. A method of identifying a compound that modulates intermolecular interactions between a target protein and a modifier comprising the steps of:
- a) identifying a cavity on said target protein proximal to a
- 5 functionally critical site of said target protein that is involved in intermolecular interactions with said modifier;
- b) calculating dimensions of said cavity and mapping chemical and/or electrostatic properties of said cavity;
- c) identifying compounds that contain functional groups that can be
- 10 accommodated by said cavity;
- d) testing said compounds in an *in vitro* assay to detect a compound which modulate intermolecular interactions between said target protein and said modifier.
2. A pharmaceutical composition comprising:
- 15 a) a pharmaceutically acceptable carrier or diluent; and
- b) a therapeutically effective amount of a compound having a structure selected from the group consisting of Formulae I-XIX.
3. A method of treating an individual suffering from an inflammatory condition comprising the step of administering to said individual a therapeutically
- 20 effective amount of a compound having a structure selected from the group consisting of Formulae I-III.
4. A method of treating an individual suffering from an undesirable immune response or immunological condition comprising the step of administering to said individual a therapeutically effective amount of a compound having a structure selected
- 25 from the group consisting of Formulae I-VI.
5. A method of treating an individual suffering from a bacterial infection comprising the step of administering to said individual
- a) penicillin or a penicillin derivative antibiotic, and

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b) a compound having a formula selected from the group consisting of Formulae VII-XIX.

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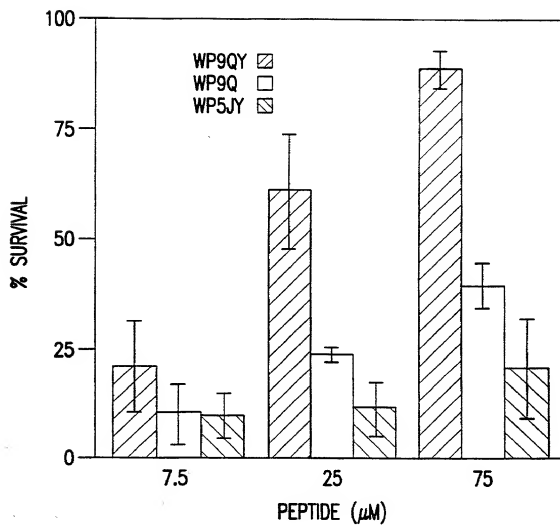


FIG. 1B

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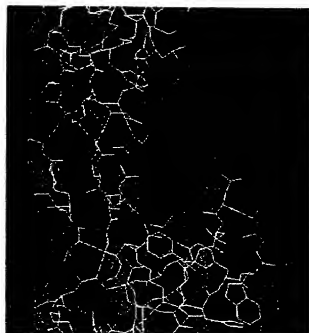


FIG.2A

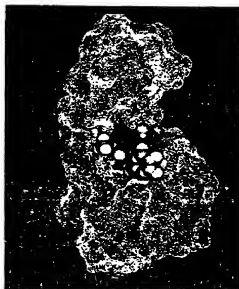


FIG.2B

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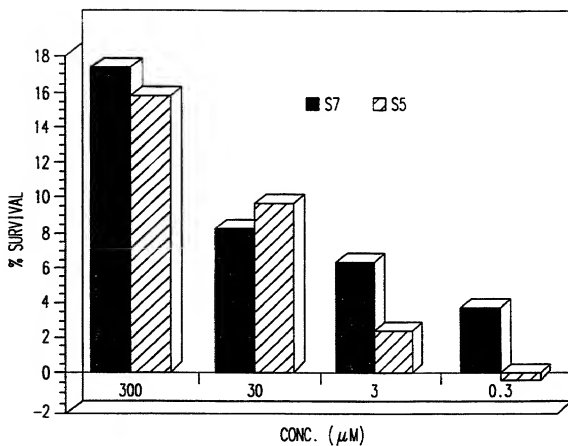


FIG. 2C

DOCKET NO. UPN-3963

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

Ramachandran MURALI and Mark I. GREENE

Group Art Unit: not yet known**International Appln. No.:** PCT/US99/15062**Examiner:** not yet assigned**International Filing Date:** 01 July 1999

For: CAVITY INDUCED ALLOSTERIC
MODIFICATION OF
INTERMOLECULAR INTERACTIONS
AND METHODS OF IDENTIFYING
COMPOUNDS THAT EFFECT THE
SAME

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name; and

I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a



Utility Patent



Design Patent

is sought on the invention, whose title appears above, the specification of which:



is attached hereto.



was filed on 01 July 1999 in the PCT/US/RO under International
Serial No. PCT/US99/15062 .



said application having been amended on _____ .

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

00726647-021201

I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to be material to the patentability of this application in accordance with 37 CFR § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a-d) of any **foreign application(s)** for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of any application on which priority is claimed:

Priority Claimed (If X'd)	Country	Serial Number	Date Filed
<input type="checkbox"/>	_____	_____	_____
<input type="checkbox"/>	_____	_____	_____
<input type="checkbox"/>	_____	_____	_____
<input type="checkbox"/>	_____	_____	_____

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to be material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Serial Number	Date Filed	Patented/Pending/Abandoned
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below:

Serial Number

Date Filed

60/091,431

01 July 1998

60/133,435

11 May 1999

I hereby appoint the following persons of the firm of **WOODCOCK WASHBURN KURTZ MACKIEWICZ & NORRIS LLP**, One Liberty Place - 46th Floor, Philadelphia, Pennsylvania 19103 as attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

Mark DeLuca

Reg. No. 33,229

Reg. No. _____

Address all telephone calls and correspondence to:

Mark DeLuca

WOODCOCK WASHBURN KURTZ

MACKIEWICZ & NORRIS LLP

One Liberty Place - 46th Floor

Philadelphia PA 19103

Telephone No.: **(215) 568-3100**

Facsimile No.: **(215) 568-3439**

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

00720647-073731

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City/State of Actual Residence: Drexel Hill, Pennsylvania	

Name: <u>Mark I. GREENE</u>	<u>Mark I. Greene</u> Signature Date of Signature: <u>6/27/2001</u> Citizenship: <u>United States</u>
Mailing Address: 300 Richters Mill Road <u>Penn Valley</u> , Pennsylvania 19072 PA	
City/State of Actual Residence: Penn Valley, Pennsylvania	